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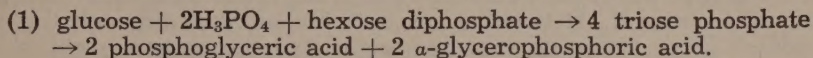
THE ROLE OF PHOSPHOGLYCERIC ACID IN THE DISSIMILATION OF GLUCOSE BY BACTERIA OF THE *ESCHERICHIA-AEROBACTER* GROUP

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From the Department of Bacteriology, Iowa State College

Accepted for publication June 16, 1936

In 1933 Embden, Deuticke, and Kraft upset existing theories of muscle glucolysis by proposing the following initial stages:



This scheme has been confirmed and somewhat modified by Meyerhof and Kiessling (1933), and applied in principle to alcoholic fermentation (1934). The same authors (1935) have presented evidence that methylglyoxal is not an intermediate in glucose dissimilation but arises as a stabilization product from an intermediate triose. In the light of the findings on muscle and yeast glucolysis, it is of interest to reinvestigate the mechanism of bacterial dissimilation, particularly as present schemes generally accept methylglyoxal as the key intermediate.

Recently Tikka (1935) has suggested that the initial steps of glucose breakdown by *Escherichia coli* may follow the same path as Embden's scheme for muscle. Although Tikka showed that fresh living cells of *Esch. coli* dissimilated phosphoglyceric acid and α -glycerophosphate, he was unable to isolate either compound. Werkman, Zoellner, Gilman and Reynolds (1935) have already reported the isolation of phosphoglyceric acid from glucose by resting cells of *Citrobacter freundii*. The isolation of phosphoglyceric acid from the dissimilation of glucose by *Esch. coli* and *Aerobacter indologenes* is reported in this communication. The evidence suggests strongly that phosphoglyceric acid plays an essential role in bacterial dissimilation, corresponding to its function in muscle and yeast glucolysis.

EXPERIMENTAL

The isolation of the phosphoglyceric acid formed by *Escherichia coli* was accomplished by the method of Neuberg and Kobel (1933) with certain modification. In a small flask were placed 17 gm. fresh cell paste (prepared by super-centrifuging the cells from a glucose-peptone broth), 25 cc. 0.67 M phosphate buffer (pH 6.8), 32 cc. 20 per cent glucose, 0.5 cc. 1 per cent MgCl_2 , 5 cc. H_2O and 3 cc. toluol. The mixture was warmed to 37° C., 0.5 cc. removed for phosphate determination and the flask placed in a 37° C. incubator. The initial or phosphorylation period was terminated after 3 hours; 25 cc. of 2 per cent acetaldehyde and 4 cc. of 0.2 M sodium fluoride were added. Again a 0.5 cc. sample was taken for phosphate determination and the mixture returned to the incubator. The second period was completed in 3.5 hours and the mixture was centrifuged to remove the cells. The inorganic phosphate was precipitated by

addition of 12 cc. of 20 per cent Mg acetate to the solution, and made alkaline with NH_4OH . Ten cc. of glacial acetic acid and 5 cc. of 50 per cent Ba acetate were added to the filtrate. The resulting precipitate was immediately removed by centrifuging and from the supernatant liquid 0.226 gm. of the barium salt of phosphoglyceric acid slowly crystallized after 36 hours at 5°C . The phosphate determinations were made colorimetrically by the method of Kuttner and Lichtenstein (1930). The results are given in the accompanying table.

For the isolation of phosphoglyceric acid from *Aerobacter indologenes*, substantially the same procedure was used. Only 20 cc. of 20 per cent glucose were added and the initial mixture made up to 60 cc. At the end of the first period 15 cc. of 2 per cent acetaldehyde and 2 cc. of 0.2 M NaF were added and 0.257 gm. of the crude phosphoglyceric acid salt was obtained. It is important to point out that although the procedures given above do not necessarily give the optimal yield of the ester, small variations in the method may produce large variations in yield, making the preparation difficult and uncertain.

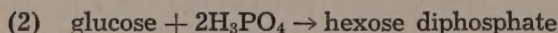
Determination of inorganic phosphate in reaction mixtures
(calculated as mgms. P/cc.)

Trial	Organism	Phosphate in reaction mixture			Phosphate uptake		
		Original	End of first period	Final	First period	Second period	Total
54	<i>Esch. coli</i>	5.38*	4.33	3.52	1.05	0.81	1.86
49	<i>A. indologenes</i>	5.97*	4.73	5.03	1.24	-0.30	0.94

* Corrected for dilution occurring by addition of acetaldehyde and fluoride solutions.

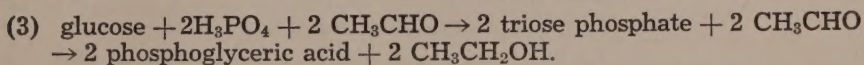
The crude phosphoglyceric acid salt was purified by solution in seventy times its weight of 0.05N HCl and reprecipitated by addition of two volumes of 95 per cent ethyl alcohol. The barium salt appeared as small white leaflets, giving the mixture a translucent sheen. The optical rotation of a 2.7 per cent solution is -0.35° ($[\alpha]_{28^\circ\text{D}} = -13.0$). Neuberger obtained -0.36° for phosphoglyceric acid from yeast. The behavior of the compound was observed to be identical with that of the barium salt obtained from *Citrobacter*, *Propionibacterium*, and yeast.

The mechanism of formation of the phosphoglyceric acid may be represented as follows. During the first period phosphorylation of the glucose occurs accounting



for the phosphate uptake shown in the initial period. Meyerhof and Kiessling (1934) believe that in alcoholic fermentation the presence of the hexose diphosphate is necessary for the breakdown of glucose to proceed

at a normal rate. After formation of the hexose ester, presumably some phosphoglyceric acid may be formed by reaction 1. However, as there is no fluoride present, the phosphoglyceric acid formed is broken down. It is evident that as soon as sufficient hexose diphosphate is produced, the first period should be ended and the sodium fluoride and acetaldehyde added. In the presence of acetaldehyde and hexose diphosphate the main reaction for the formation of the ester is probably



Such a reaction will result in a phosphorus uptake for the second period as shown in the table for trial 54 with *Esch. coli*. However, *A. indologenes* shows a decrease in inorganic phosphate during the second period. Apparently the dephosphorylation of phosphate esters formed in the first period proceeded more rapidly than the reaction given above.

It may be objected that the isolation of phosphoglyceric acid from the toluol treated organisms does not give us a true picture of an unpoisoned normal fermentation. This objection must be considered because at the present time the exact function of the toluol is unknown. Neuberg and Kobel explain its action as that of a cell plasmolyzing agent. It may be safely said that the organisms possess the necessary enzyme equipment for the production of phosphoglyceric acid. The isolation, coupled with the fact that Tikka has shown *E. coli* able to effect its breakdown to normal end products, presents strong evidence that phosphoglyceric acid is a normal intermediate in fermentation by *Escherichia* and *Aerobacter*. It is apparent that the mass of evidence for the Embden-Meyerhof scheme of muscle glycolysis may equally well apply to bacterial dissimilation.

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OXIDATIVE DEGRADATION OF SILK¹

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Silk is exposed to oxidants of the atmosphere and certain processes of bleaching, dyeing, and printing. A review of the literature has disclosed a great number of empirical recipes for bleaching silk with permanganates, peroxides, perborates, percarbonates, and persulfates but no quantitative information about the effect of these oxidants on its composition and physical properties. Wild silk fibroin has been characterized as more resistant than silk fibroin to oxidizing mordants (13); three-volume as well as ten-volume hydrogen peroxide has been recommended for bleaching (17, 10), though described as yellowing wild silk at a high temperature (2); ozone has been found to make silk yellow, harsh, and lusterless (4); and the use of sodium perborate, recommended for the oxidation of Indanthrene dyeings on silk (21), has been considered hazardous for weighted silk (16). The effects of weathering and storage on the composition and mechanical performance of silk have been investigated but without separation of the factors involved. The effectiveness of reductants in retarding degradation of weighted silks ascribed to oxidation has been questioned (15, 24, 23, 9, 3, 20).

Quantitative data of the effect of hydrogen peroxide and aqueous potassium permanganate in ten hours at 40° C. on the weight, nitrogen, ash, and wet strength of wild silk fibroin, silk fibroin, black iron-weighted, white lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks of typical commercial quality are reported in this study.

EXPERIMENTAL PROCEDURE

PREPARATION OF FABRICS

Silk crêpe, plain-woven in the gum, was boiled one hour in one hundred volumes of ten per cent neutral olive-oil soap, rinsed, boiled in another bath of the soap and then in water three times for 15 minutes each. Plain-woven pongee of wild silk was boiled one hour in water, rinsed, and again boiled in water for 15 minutes, and thoroughly rinsed. These fabrics were cut for analysis, continuously extracted with anhydrous ether for 18 hours and, with the exception of samples for physical analysis, dried at 105° to 110° C. until successive weighings with tare checked within half a milligram. The plain-woven weighted silks were cut for analysis or dried to constant weight without any pretreatment.

ANALYSIS OF FABRICS

The seven fabrics were conditioned for four or more hours at 70° ± 2° F. and 65 ± 2 per cent R. H. before analysis for weight, thick-

¹ Journal Paper No. J269 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 262.

² The authors wish to thank Miss Florence Barr for checking some of the analyses.

TABLE 1. Analysis of the silk fabrics

Silk	Weight oz. per sq. yard	Thick- ness inch	Yarn						Twist* number per inch filling
			Number per inch		Percentage of fabric		Count		
			warp	filling	warp	filling	warp	filling	
A. Iron-weighted silk crêpe	3.00	0.0066	160	74	69.0	31.1	48.7	46.9	62 (2) **
B. Lead-weighted silk crêpe	3.17	0.0065	127	74	60.6	37.8	37.4	35.3	75 (5)
C. Tin-weighted silk crêpe	2.86	0.0063	155	72	65.1	34.9	44.8	44.8	84 (4)
D. Tin-lead-weighted silk crêpe	3.15	0.0065	180	79	71.4	28.5	45.4	48.5	62 (3)
E. Zinc-weighted silk crêpe	2.86	0.0062	170	70	73.0	26.0	51.9	48.5	60 (2)
F. Silk crêpe	2.20	0.0067	246	109	59.0	40.3	127.8	88.2	0
G. Wild silk pongee	1.25	0.0042	76	77	45.3	53.7	71.7	78.0	

* Two left-twisted filling yarns alternated with two right-twisted filling yarns; the warp yarns were not measurably twisted.

** Average deviations.

TABLE 1. (Continued)

Silk	Ash percentage of yarn warp filling	Breaking strength				Elongation at breaking load			
		Of conditioned fabrics		Of wet fabrics		Of conditioned fabrics		Of wet fabrics	
		pounds per inch		percentage of dry		percentage		percentage	
		warp	filling	warp	filling	warp	filling	warp	filling
A.	42.5	34 (0.6) **	12 (0.2) **	97	75	10	7	25	34
B.	44.5	30 (0.3)	12 (0.7)	57	58	7	7	16	23
C.	54.2	38 (1.0)	13 (0.5)	53	46	10	9	35	23
D.	52.6	42 (0.3)	13 (0.5)	57	46	10	7	35	28
E.	48.7	40 (0.4)	14 (0.4)	58	50	10	8	35	31
F.	49.9	38 (0.9)	32 (0.4)	92	81	34	37	56	55
G.	53.7	21 (0.4)	23 (1.3)	81	43	30	30	38	37

TABLE 1. (Continued)

Silk	Aluminum	Ash	Iron	Lead	Nitrogen	Phos- phorus	Silica	Tin	Water extract	Weight- ing	Zinc
							<i>percentage of fabric</i>				
A.		43.7	15.11	12.21	8.16	0.56	12.52		10.9	70.6	
B.	trace	44.2			9.17	0.91	20.30		8.8	55.3	
C.		52.9			9.26	0.45	20.49	8.80	7.2	61.3	
D.	trace	50.3		9.10	8.46	1.06	14.91	8.66	4.4	60.8	
E.	0.85	51.9			8.10	0.57	15.07		4.6	63.5	
F.		0.3			18.59						11.12
G.		0.6			18.25						

ness, yarns, breaking strength by the one-inch-strip method, and elongation at breaking load (1, 5).

The nitrogen of four-gram samples of silk fibroin or wild silk fibroin was determined by the Kjeldahl-Gunning-Arnold method (11), that of the weighted silks by the Kjeldahl method (14).

Each value for water extract and weighting is the average of four determinations made with five-gram samples (25). Two four-gram samples of yarn were dried to constant weight at 105° C. and ignited until constant in an electric furnace at dull red heat; the ash of the iron-weighted silk was treated with nitric acid and again ignited. For each fabric four five-gram samples were similarly analyzed for total ash. The phosphorus of the ash was determined gravimetrically (6A) and the silica as loss in weight upon treatment with hydrofluoric acid (8). The residues from hydrofluoric-acid treatment of the ash were then further analyzed; that of the iron-weighted silk was fused with potassium pyrosulfate before solution in dilute sulfuric acid for volumetric determination of iron (22b); the lead of the lead-weighted and tin-lead-weighted silks was weighed as sulfate (22c) and the tin of the tin-weighted and tin-lead-weighted silks as stannic oxide (6b); the aluminum of the zinc-weighted silk was weighed as oxide (22a) and the zinc was determined volumetrically (22d). Analyses were made with separate samples except in cases of tin and lead of the tin-lead-weighted and aluminum and zinc of the zinc-weighted silks.

Throughout this study the average of three or more chemical determinations has been expressed as percentage of textile dried to constant weight at 105° to 110° C. and weighed with tares. The fabrics are described by analysis in table 1.

TREATMENT OF FABRICS WITH HYDROGEN PEROXIDE

Four grams of silk fibroin or wild silk fibroin were immersed in 200 cc. of 0.005 *M* sodium carbonate, 1.0900 *M* or 2.1800 *M* hydrogen peroxide (27) made 0.005 *M* with respect to sodium carbonate, in a 250-cc. stoppered Erlenmeyer flask in a water bath at 40° ± 0.1° C. for ten hours and then washed in water until the rinse no longer reduced permanganate. After drying at room temperature the residual silks were analyzed for nitrogen or again dried to constant weight.

TABLE 2. *Effect of hydrogen peroxide in ten hours at 40° C. on the weight, nitrogen, and wet strength of silk fibroin and wild silk fibroin*

Hydrogen peroxide	Sodium carbonate	Weight		Nitrogen		Breaking strength of wet warp	
		Silk	Wild silk	Silk	Wild silk	Silk	Wild silk
		percentage of original fabric				pounds per inch	
<i>molality</i>	<i>molality</i>						
0	0.005	99.8	99.0	18.61	18.16	26	16
1.0900	0.005	96.9	97.6	18.06	17.95	17	12
2.1800	0.005	93.3	96.3	17.10	17.65	< 1	11

Ten warp strips for test of strength, treated in the same way but without initial drying, were tested wet after rinsing. The weighted silks were

unchanged in wet strength after exposure to high concentrations of hydrogen peroxide for ten hours at 40° C., although the peroxide was decomposed by the weighting (12).

TREATMENT OF FABRICS WITH AQUEOUS POSTASSIUM PERMANGANATE

Approximately four grams of wild silk fibroin were immersed in 200, 500 or 1000 cc. of 0.0237 *M* potassium permanganate (7) ten hours at 40° ± 0.1° C., freed of manganese dioxide in two hours by 0.05 *M* sodium hydrogen sulfite, and washed in water until the rinse failed to reduce permanganate. After drying at room temperature the residues were either analyzed for nitrogen or dried to constant weight. Because of decrease in weight and linear decrease in nitrogen of wild silk fibroin with increasing volume of permanganate (table 3), fifty volumes of permanganate per gram of fabric were used in further treatments.

TABLE 3. *Effect of volume of aqueous potassium permanganate on the weight and nitrogen of wild silk fibroin in ten hours at 40° C.*

Potassium permanganate		Weight	Nitrogen
cc. 0.0237 <i>M</i>	gram per gram fibroin	percentage of original fabric	
(200 cc. water)	0	99.5	18.24
200	0.466	96.4	17.86
	0.493	96.6	17.86
	0.512	96.5	17.77
	0.572	96.0	17.76
500	1.343	94.0	17.13
	1.447	92.7	17.02
1000	2.358	83.8	15.73
	2.401	83.0	15.51

In the case of each of the fabrics approximately five grams were immersed for ten hours at 40° ± 0.1° C. in fifty volumes of water or permanganate in a 500-cc. stoppered Erlenmeyer flask. The residual fabric was freed of manganese dioxide in 30 minutes by fifty volumes of 0.05 *M* sodium hydrogen sulfite and washed in water until the rinse did not reduce permanganate; sodium hydrogen sulfite under these conditions had no effect on silk fibroin or wild silk fibroin. Wet warp strength was determined at once, nitrogen after drying at room temperature, and ash after drying to constant weight.

DISCUSSION OF RESULTS

One factor of the greater degradation of the silk fibroins by permanganate (tables 5 and 7) than by peroxide (table 2) is the dissipation of the alkaline hydrogen peroxide by rapid evolution of oxygen (blank determinations testing 1.0900 *M* and 2.1800 *M* decreased to 0.2463 *M* and 0.7666 *M*, respectively, in ten hours at 40° C.); another and probably a large factor is the equimolal concentration of potassium hydroxide resulting from complete decomposition of the permanganate (26, 19).

The weight and ash of the residual weighted silks (table 4) show that little weighting was removed by the permanganate.

TABLE 4. *Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the weight, ash, and nitrogen of the weighted silks*

Potassium permanganate molarity	Iron-weighted silk			Lead-weighted silk			Tin-weighted silk		
	Weight	Ash	Nitrogen	Weight	Ash	Nitrogen	Weight	Ash	Nitrogen
0	96.5	40.2	8.08	percentage of original fabric					
0.0030	96.0	40.2	8.02	93.0	44.1	9.08	93.1	51.4	8.64
0.0060	95.0	39.6	7.47	92.9	40.9	8.94	92.2	51.2	8.46
				88.7	40.1	8.26	92.0	50.0	7.70

TABLE 4. (Continued)

Potassium permanganate molarity	Tin-lead-weighted silk			Zinc-weighted silk		
	Weight	Ash	Nitrogen	Weight	Ash	Nitrogen
		percentage of original fabric				
0	96.0	49.0	8.50	96.0	50.1	7.79
0.0030	95.7	48.6	8.36	94.0	50.0	7.39
0.0060	94.7	48.4	7.66	89.4	49.5	6.85

TABLE 5. *Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the weight, ash, and nitrogen of silk fibroin and wild silk fibroin*

Potassium permanganate molarity	Silk fibroin			Wild silk fibroin		
	Weight	Ash	Nitrogen	Weight	Ash	Nitrogen
	percentage of original fabric					
0	99.4	0.3	18.56	99.5	0.7	18.25
0.0030	98.7	0.5	18.21	99.2	0.7
0.0060	98.7	0.6	18.22	99.1	0.8	18.23
0.0083	98.6	0.7	18.11	98.4	1.1	18.12
0.0167	97.9	0.8	17.72	98.4	1.3	17.90
0.0237	97.7	1.0	17.68	97.9	1.4	17.81

TABLE 6. *Percentage of fibroin* dissolved in ten hours at 40° C. by fifty volumes of aqueous potassium permanganate*

Potassium permanganate gram per gram fibroin	Fabric						
	A	B	C	D	E	F	G
0.0969	6.0	9.0	10.0	8.7	9.3	3.4	1.3
0.1017	6.6		10.9	9.5	10.1	3.5	1.4
0.1035	6.9			9.8	10.5	3.6	1.4
0.1090	7.6				11.4	3.8	1.5
0.1129					12.1	3.9	1.6

* Calculated from nitrogen; values in italics are experimental, the others interpolated.

TABLE 7. *Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the wet warp breaking strength of the fabrics*

Potassium permanganate molarity	Fabric						
	A	B	C	D	E	F	G
	pounds per inch						
0.0000	32	20	24	21	21	27	15
0.0030	30	10	17	9	17	12	6
0.0060	23	9	<1	6	<1	10	4
0.0083	<1	<1		<1		9	3
0.0167						6	2
0.0237						<1	<1

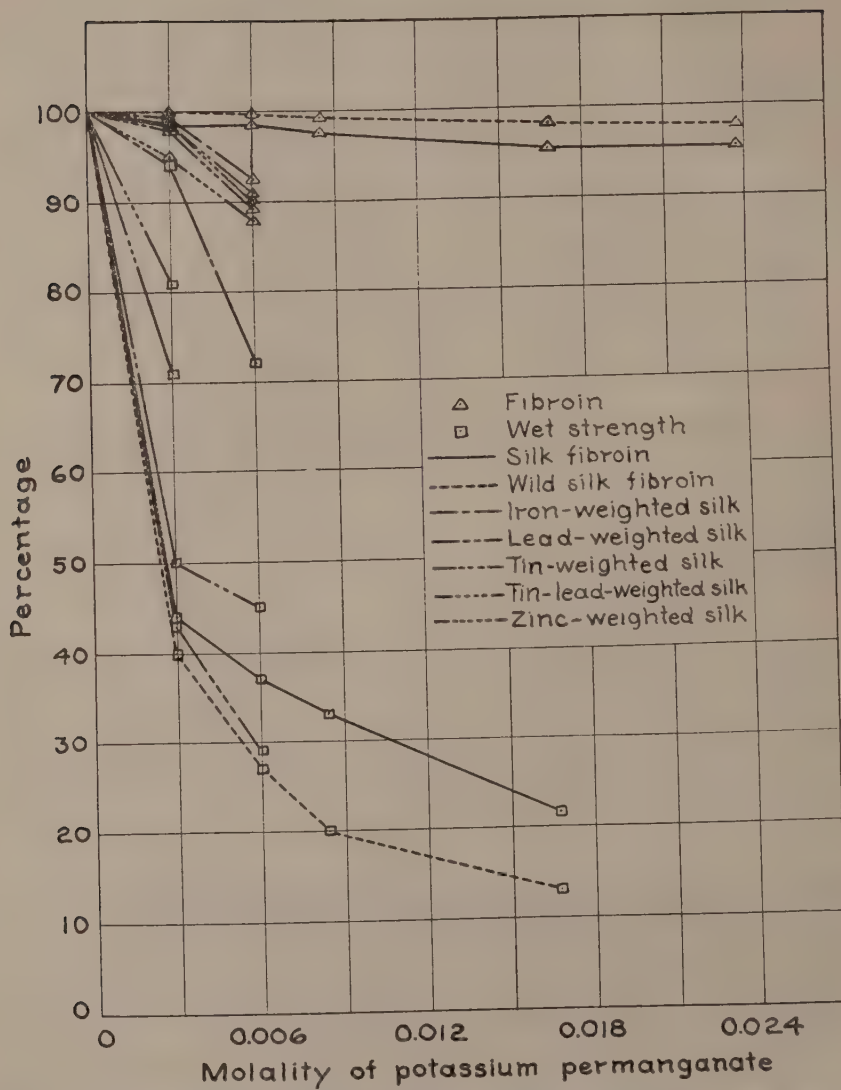


Fig. 1. Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the fibroin and wet strength of the fabrics.

Although dilution of fibroin by weighting increases its oxidative degradation to soluble forms of nitrogen (tables 4, 5 and 6), the loss and rate of loss in wet strength with increasing concentration of oxidant exceed the loss and rate of loss of fibroin (Fig. 1). The pattern of the mechanical performance of the iron-weighted, lead-weighted, tin-weighted, and zinc-weighted silks during oxidative degradation shows their wet strengths as high or higher than that of silk up to the time of their complete degradation. This rapid loss of strength is similar to that which occurs in dry cleaning and laundering lead-weighted and tin-lead-weighted silks (18).

SUMMARY

1. The course of the degradation of silk fibroin and wild silk fibroin by hydrogen peroxide and of silk fibroin, wild silk fibroin, black iron-weighted, white lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks by aqueous potassium permanganate in ten hours at 40° C. has been followed by analysis of the residual plain-woven fabrics for weight, nitrogen, ash, and wet warp strength.

2. The weighted silks were unchanged in wet strength by high concentrations of hydrogen peroxide; wild silk fibroin has been shown more stable than silk fibroin to 1.09 and 2.18 M hydrogen peroxide.

3. It has been shown that degradation of the fabrics by potassium permanganate is much greater than by hydrogen peroxide; that nitrogen of wild silk fibroin is a decreasing linear function of the volume of potassium permanganate at a given concentration; that nitrogen of silk fibroin and nitrogen of wild silk fibroin are almost linear functions of the concentration of oxidant; that dilution of fibroin by weighting results in greater solution of its nitrogen upon oxidative degradation; and that loss and rate of loss in wet strength with increasing concentration of permanganate, different for the various fabrics, are greater than loss and rate of loss of fibroin.

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DEGRADATION OF FIVE WEIGHTED SILK FIBROINS BY STEAM¹

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A recent editorial traces the tremendous annual losses in the drying of textiles to the blind acceptance of meaningless "safe drying temperature limits," regardless of the textile, its regain or chemical processing, and time of steaming (12). Although steam is used in lustering (9), conditioning (11), and aging dyed and printed weighted silks (1, 2, 3, 4, 5, 8, 10) as well as in their maintenance during use, there is little description of the effect of steam on the composition and properties of weighted silks.

The 48 per cent loss of dry strength upon steaming a silk 46 hours at 100° C. and the 55 per cent loss of this same silk when tin-weighted, compared to corresponding losses of 25 and 22 per cent upon drying the silks the same time at the same temperature, suggest that weighting and its hydrolytic products are factors of the greater deterioration by steam (6).

We have measured the relative resistance to degradation by steam of plain-woven crêpes of silk fibroin, iron-weighted, lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silk fibroins by analysis of the residual fabrics for weight, ash, nitrogen, and wet strength.

EXPERIMENTAL PROCEDURE

Five four-gram samples of one of the fabrics (previously defined analytically 7), or five one-inch-warp strips were steamed at one time in an autoclave equipped with an accurate pressure gauge and thermometer; the fabrics, attached by silk thread to glass rods laid across the top, were hung in a four-liter Pyrex beaker having a low exit for steam and were protected by an inverted watch glass from any liquid which had flowed across metal. In one series of tests the fabrics were steamed at 0, 8, 18, 28, 33, and 38 pounds for one hour; in another series steaming at eight pounds was continued for two, three, four, and five hours. The residual fabric was rinsed in water eight times to remove soluble derivatives; wet strength was determined at once, nitrogen after drying at room temperature, and ash after drying the weighted silks to constant weight (7). Each value reported for wet strength is the average of ten determinations; values for weight, ash, and nitrogen are the averages of four closely agreeing determinations expressed as percentage of the original fabric dried at 105° to 110° C. until successive weighings with tare checked within half a milligram.

DISCUSSION OF RESULTS

The greater degradation of the silk fibroin than of a heavier crêpe formerly described (13) shows that increasing the yardage per weight of

¹ Journal Paper No. J361 of the Iowa Agricultural Experiment Station, Ames, Iowa.

² The authors wish to thank Professor C. C. Major for calibration of the steam gauge.

TABLE 1. *Effect of steam on the weight, ash, and nitrogen of the fabrics*

Temperature °C.	Pressure of steam pounds per sq. in.	Time hour	A. Iron-weighted silk		B. Lead-weighted silk		C. Tin-weighted silk				
			Weight	Ash	Nitrogen	Weight	Ash	Nitrogen	Weight	Ash	Nitrogen
percentage of original fabric											
100.0	0	1	99.3	43.6	8.17	99.0	43.8	9.05	99.1	51.5	9.22
112.6	8	1	99.2	43.6	8.15	98.9	43.7	9.05	98.8	51.3	9.09
		2	98.0	43.1	8.09	96.0	42.7	8.88	96.3	50.1	8.38
		3	95.6	41.2	7.67	93.8	41.7	8.04	93.9	48.4	8.12
		4	93.5	40.0	7.53	89.9	40.8	6.57	92.1	47.2	7.74
		5	93.3	39.9	7.31	88.9	40.3	5.63	89.2	46.0	7.47
124.1	18	1	98.0	41.3	8.09	95.9	41.1	8.86	95.6	50.1	8.16
132.9	28	1	95.4	40.3	7.64	88.8	40.1	5.64	89.9	47.3	7.55
140.2	38	1	92.8	38.3	7.28	86.8	38.2	5.08	88.0	46.2	5.99

TABLE 1. (Continued)

Temperature °C.	Pressure of steam pounds per sq. in.	Time hour	D. Tin-lead-weighted silk		E. Zinc-weighted silk		F. Silk fibroin			
			Weight	Ash	Nitrogen	Weight	Ash	Nitrogen	Weight	Nitrogen
percentage of original fabric										
100.0	0	1	99.2	51.6	8.45	98.9	50.1	8.08	99.7	18.61
112.6	8	1	99.0	51.0	8.42	98.8	50.1	8.05	99.4	18.37
		2	96.9	49.4	8.34	98.2	50.1	7.98	99.3	18.29
		3	93.2	47.7	7.68	97.0	50.2	7.03	99.1	18.23
		4	92.8	47.2	7.54	90.1	49.0	6.08	98.4	18.19
		5	91.6	46.9	7.39	85.9	47.9	5.32	98.0	18.16
124.1	18	1	96.8	49.3	8.29	95.9	50.0	7.80	98.9	18.25
132.9	28	1	93.6	47.7	7.54	88.0	48.4	5.91	97.4	18.15
140.2	38	1	90.8	45.3	7.15	84.7	46.7	4.96	96.4	17.77

TABLE 1. (Continued)

Temperature °C.	Pressure of steam pounds per sq. in.	Time hour	D. Tin-lead-weighted silk		E. Zinc-weighted silk		F. Silk fibroin			
			Weight	Ash	Nitrogen	Weight	Ash	Nitrogen	Weight	Nitrogen
			percentage of original fabric							
100.0	0	1	99.2	51.6	8.45	98.9	50.1	8.08	99.7	18.61
112.6	8	1	99.0	51.0	8.42	98.8	50.1	8.05	99.4	18.37
		2	96.9	49.4	8.34	98.2	50.1	7.98	99.3	18.29
		3	93.2	47.7	7.68	97.0	50.2	7.03	99.1	18.23
		4	92.8	47.2	7.54	90.1	49.0	6.08	98.4	18.19
		5	91.6	46.9	7.39	85.9	47.9	5.32	98.0	18.16
124.1	18	1	96.8	49.3	8.29	95.9	50.0	7.80	98.9	18.25
132.9	28	1	93.6	47.7	7.54	88.0	48.4	5.91	97.4	18.15
140.2	38	1	90.8	45.3	7.15	84.7	46.7	4.96	96.4	17.77

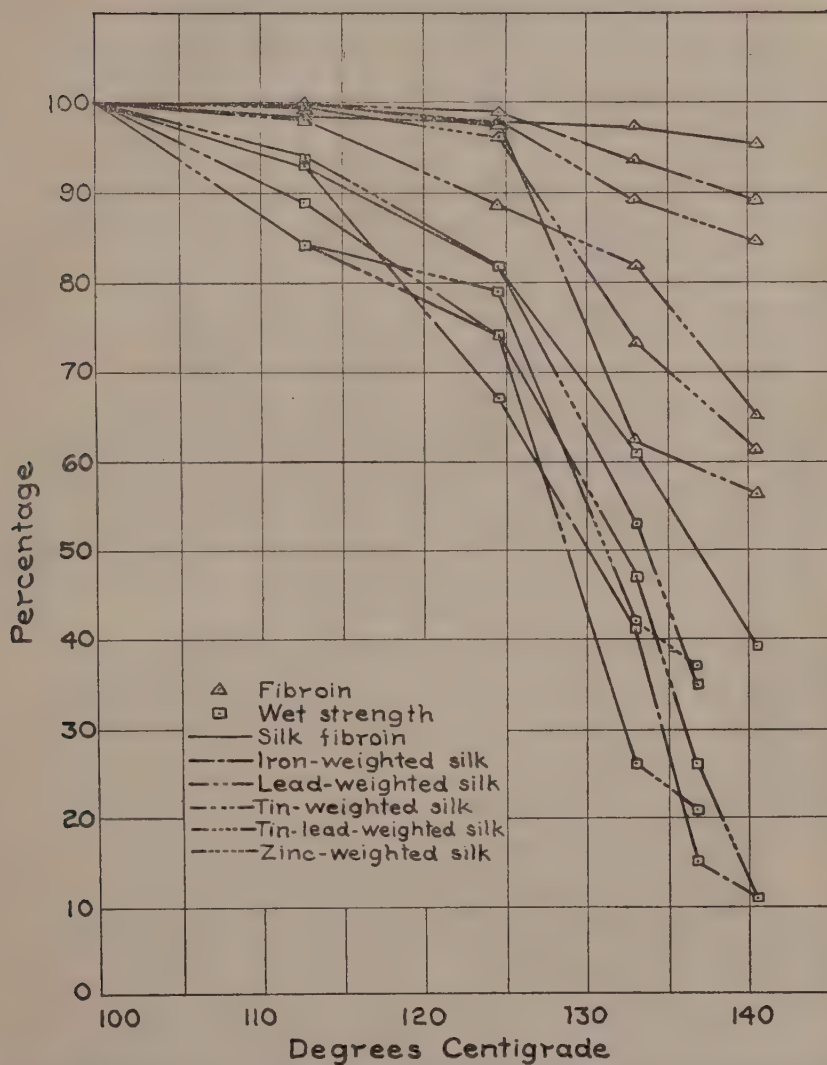


Fig. 1. Effect of steam at 0, 8, 18, 28, 33, and 38 pounds per square inch on the fibroin and wet warp breaking strength of the fabrics.

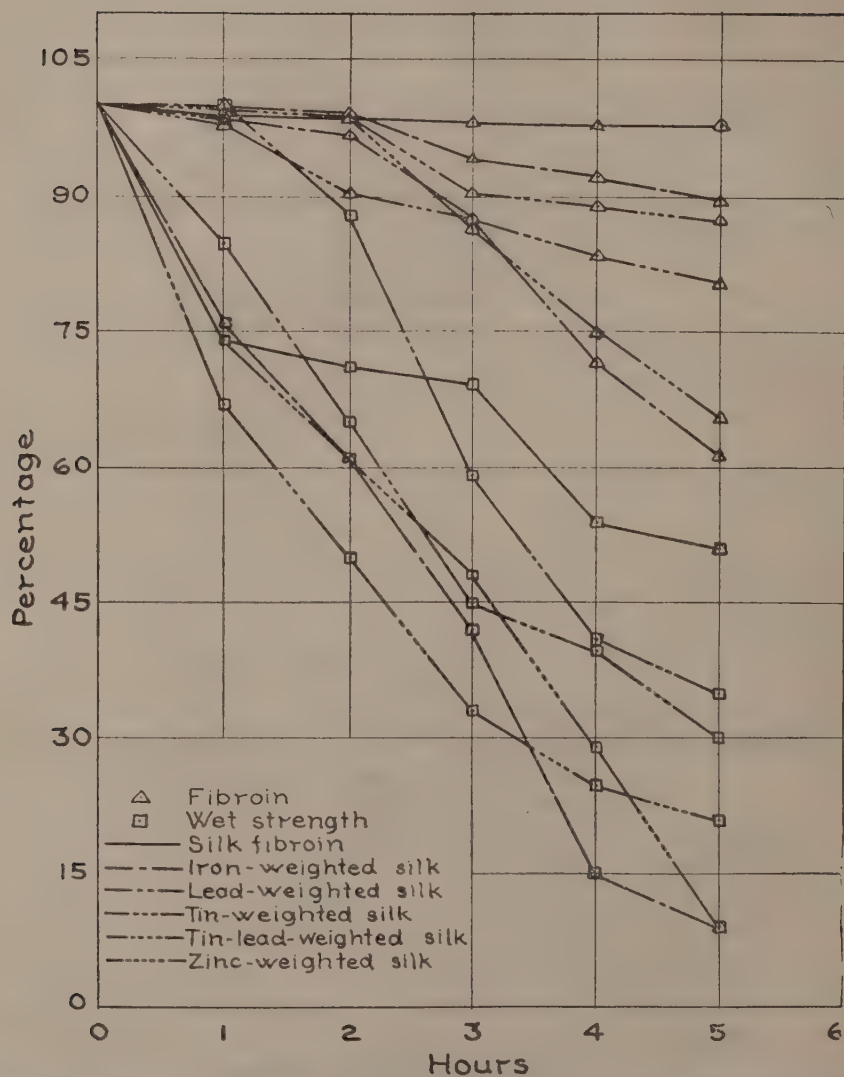


Fig. 2. Effect of steam at 112.6° C. on the fibroin and wet warp breaking strength of the fabrics.

TABLE 2. *Effect of steam on the wet warp breaking strength of the fabrics*

Temper- ature	Pressure of steam	Time	Fabrics					
			A	B	C	D	E	F
°C.	pounds per sq. in.	hour	pounds per inch					
100	0	1	27	19	19	17	19	28
112.6	8	1	25	17	16	16	17	26
		2	20	15	13	12	14	25
		3	14	10	9	8	11	24
		4	5	7	8	6	5	19
		5	3	6	6	5	2	18
124.1	18	1	18	14	14	14	15	23
132.9	28	1	11	5	9	9	8	17
136.7	33	1	4	4	5	6	7
140.2	38	1	3	<1	2	<1	<1	11

silk fibroin decreases its stability to steam. This raises the question as to whether the greater loss of strength by weighted silk may not be due in large part to its lower weight of silk fibroin per yard of fabric.

Tables 1 and 2 and figures 1 and 2 show that dilution of fibroin by weighting increases its conversion to soluble derivatives by steam and that loss in wet strength, with increasing temperature or time, is greater than this loss of fibroin.

SUMMARY

1. The course of the degradation of silk fibroin and black iron-weighted, white lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silk fibroins by steam, in one hour at 0, 8, 18, 28, 33, and 38 pounds per square inch and in two, three, four, and five hours at eight pounds, has been followed by determination of the weight, ash, nitrogen, and wet strength of the residual plain-woven crêpes after thorough rinsing.

2. In one hour at 38 pounds the maximal decrease in percentage of ash, 6.3, occurred in the case of the tin-weighted silk; the zinc-weighted silk lost the most weight, 14.2 per cent.

3. The weighted silks, particularly the lead-weighted, became brown upon steaming one hour at eight pounds; the silk fibroin at 38 pounds.

4. The percentage conversion of the fibroin to soluble forms of nitrogen, in (a) one hour at 38 pounds and (b) five hours at eight pounds, has been shown of the order: lead-weighted (a, 43.9; b, 38.6), zinc-weighted (a, 38.6; b, 34.3), tin-weighted (a, 35.0; b, 19.3), tin-lead-weighted (a, 15.4; b, 12.6), iron-weighted (a, 10.9; b, 10.4), and silk fibroin (a, 4.5; b, 2.3). With increasing pressure, this conversion of the fibroin was greatest between 18 and 28 pounds, except that the tin-weighted silk lost most between 28 and 38 pounds; at eight pounds the greatest loss occurred between two and three hours, except in the case of the lead-weighted silk which lost most between four and five hours.

5. Although dilution of fibroin by weighting increases the conversion of its nitrogen to soluble forms by steam, the loss in wet strength,

with increasing temperature or time, exceeds the loss of fibroin. The tin-weighted silk alone withstood steam at a pressure of 18 pounds for one hour as well as silk fibroin; however, this weighted silk and the lead-weighted and zinc-weighted silks showed no measurable wet strength after one hour at 38 pounds. All the weighted silks withstood a pressure of eight pounds for one hour as well as silk fibroin, but upon continued steaming suffered decidedly greater losses.

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THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD), AND ITS GREGARINE PARASITES¹

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Accepted for publication June 16, 1936

PART ONE

METHODS AND OBSERVATIONS IN REARING THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD) (THYSANURA)

The apterygotous insects have been little used as experimental animals. The use of the common household lepidoptera, the firebrat and the silverfish, is suggested. The firebrat is the larger and more tractable of the two, and it has the remarkable characteristic of being very thermophilic. Although fragile and slow of development, firebrats have proved excellent subjects for studies upon lepidopteran biology, insect toxicology, thermophilia and thermoplegia, and gregarine parasites. They are suggested for use in studies upon regeneration, embryology, gametogenesis, insect nutrition, and animal sociology.

At Iowa State College firebrats are found associated with the heating system which provides nooks in the buildings and tunnels where temperatures above 30° C. prevail most of the year. From about one hundred captured specimens the author (1) has reared many generations, including thousands of descendants, over a four-year period. The insects are reared in glass culture dishes containing plaited strips of paper; in moving air at 37° C. and a relative humidity of about 70 per cent, and in semi-darkness. They are fed upon rolled oats, dried lean beef, dried brewer's yeast, cane sugar, and common salt, each material being supplied separately. They can also be reared, but more slowly, upon rolled oats alone or upon a basal diet of starch, casein, and "complete salt mixture," supplemented with dried yeast. Under favorable conditions firebrats reach maximum weight in about five months. Individuals of the same age show increasing variation in their sizes as they pass beyond the early instars. There is evidence that such structures as the metanotum grow as much as nine per cent in width during the third instar. The sexes cannot be distinguished, without minute examination, until the eighth instar. The males and females are about equal in number. With regard to the sexual habits the writer agrees with Spencer (3) that copulation is absent and that the females take up spermatophores dropped by the males. Balls of sperm have been found in the genital tracts of females. There is no oviposition in colonies from which males are absent and oviposition ceases in a few days following removal of males. When hungry, firebrats are fairly tolerant of moderate light. When protected by glass from air-currents (which excite them strongly) they feed and oviposit in the normal daylight of the laboratory table. There is evidence that these insects

¹ Original thesis submitted December, 1935. Doctoral thesis number 355.

are somewhat gregarious, although they usually avoid direct contacts with each other. Fighting and cannibalism occur under stress of hunger.

PART TWO

THE TEMPERATURE RELATIONS OF THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD) (THYSANURA)

The firebrat, *Thermobia domestica* (Pack.), has been recognized as a heat-loving insect ever since it became known to science over 60 years ago. It is restricted to the vicinities of hearths, ovens, heat conduits, and to other habitats where temperatures above 30° C. prevail. In contrast to the temperature relations of the firebrat those of the larvae of the corn borer, *Pyrausta nubilalis* Hubn., are cited from unpublished studies. The borer thrives at ordinary summer temperatures near 27° C. The larvae were killed in laboratory experiments by one-hour exposure in air at a temperature of 48° C. and survived prolonged exposure to temperatures in the winter of southern Ontario. Experiments upon the firebrat were aimed at determining its maximum and minimum fatal temperatures, the range of its preferred temperatures, and its thermotactic optimum. The latter is defined as the temperature the insect is most likely to choose by thermotactic responses when offered access to a range of suitable environments differing only in temperature. A thermogradient was constructed, the principal part of which was a metal trough divided into transverse compartments separated by narrow openings. Each compartment was covered and so equipped that the insects might live in it indefinitely if suited with the temperature. When heat was applied at one end a rough, variable gradient of temperatures was obtained in the succeeding compartments. Firebrats in such a device showed that they strongly avoided temperatures outside the range of 32 to 43° C. The mean point of the distribution, which is regarded as the thermotactic optimum, was 37.5° C. In other experiments it was found that firebrats would not breed at 24.5° C. and only very slowly at 29.5° C. Oviposition occurred at 42° C. but not at 45° C. Eggs at 37° C. hatched in thirteen or more days, and at 42° C. in nine or more days. The life-cycle from egg to egg was at least four weeks shorter at 42° C. than at 37° C. In a variety of preliminary tests nymphs of the second instar and fully grown firebrats were removed from their favorable environment in the incubator and subjected to extreme temperatures. It was found that exposure to — 7° C. for one hour, or to 2° C. for less than twenty-four hours, was sufficient to kill nearly all the animals so tested. Firebrats can live for many days at 45° C. without apparent paralysis. At 47° C. adults retain the power of locomotion for at least ten hours. The nymphs are slightly less resistant to high temperatures. An exposure to 49° C. for one hour causes thermoplegia and is usually fatal, both to nymphal and to mature firebrats.

PART THREE

THE GREGARINE PARASITES OF THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD) (THYSANURA)

Two species of septate gregarines became numerous in cultures of the firebrat. As parasites of the firebrat these gregarines are convenient for continuous cultivation in the laboratory. A description, more detailed

than that previously published (2), is given for each species. Firebrats in cultures containing one or both species of gregarines seem to thrive as well as others reared gregarine-free from the egg.

Lespismatophila thermobiae Adams and Travis

As many as two hundred and fifty trophozoites have been taken from the caeca of one firebrat. The mature sporonts move toward the posterior end of the ventriculus to encyst in pairs. When the cysts are deposited by the host the gametocytes are usually hemispheroidal with a discoidal, hyaline layer between them. The latter disappears in the first day of the exogenous cycle. At 34.5° C. the cyst acquires a grayish tint on the third day, owing to the coloration of the developing sporocysts. On the fourth day the cyst becomes grayish black in color. On the fifth or sixth day it forcibly bursts, everting a ball of closely coiled spore-chains. The latter rapidly uncoil and extend until they appear as a dark, fluffy mass lying over the remnant of the ruptured cyst. At 43° C. dehiscence comes in three days. Spores retain their infective power for at least three months.

Colepismatophila watsonae Adams and Travis

The trophozoites are crowded between the peritrophic membrane and the ventricular epithelium. Sometimes they nearly block the lumen and sometimes they distend the ventricular wall. The larger sporonts frequently occupy deep cavities in the epithelium; some of these cavities have been found to extend to the basement membrane, indicating the displacement or destruction of many cells. The shortest life-cycle period determined at 33° C. is eight days. The average number of spores in a cyst is estimated, from partial counts, to be about sixteen hundred.

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THE HEAT CAPACITY OF IRON CARBIDE¹

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The free energy change accompanying the formation of a compound from its elements is a criterion of the stability of the compound. The increase in free energy, ΔF , may be found by the equation,

$$\Delta F = \Delta H - T\Delta S,$$

where H is heat content, T is absolute temperature, and S is entropy.

ΔH for the formation of Fe_3C has been determined a number of times. The results of Brodie, Jennings and Hayes, as recalculated by Yap and Liu (1), will be used in the following calculations. Values for the entropy of iron (2) and of carbon (3) have already been calculated from heat capacity data. The only additional information necessary for the calculation of ΔF is the heat capacity of Fe_3C from absolute zero up to the temperature at which ΔF is desired.

Naeser (4) has recently made some measurements on the heat capacity of iron carbide at low temperatures, but made no calculation of entropy. He obtained his data using pure Fe_3C and a water calorimeter, while in the present investigation the measurements were made on Fe_3C as it exists in steel, using the method of electrical heating in a vacuum.

MATERIAL APPARATUS, AND METHODS OF PROCEDURE

The steel samples were prepared by melting Armco iron and pure carbon in magnesia crucibles. The slugs thus produced were examined microscopically. No inclusions were observed and the carbide seemed to be present as pearlite and massive cementite. The slugs were turned down to .75 inch in diameter and about 2.31 inches in length, leaving at one end a small additional piece of metal, through which a hole was bored for suspending the sample. An Armco iron sample of the same dimensions, which was used for standardizing the calorimeter, was made from .75 inch Armco iron rod. Each slug was wound with 40 ohms of constantan wire. A coat of Bakelite lacquer was applied to give thermal contact.

The calorimeter was made entirely of Pyrex glass. It was evacuated by a mercury vapor pump in series with a mechanical pump. The sample was suspended by a silk thread from the copper wires which served as leads to the heating coil. The energy input was measured by the known resistance and the current was measured by a milliammeter.

In most cases temperature was measured by a previously calibrated thermocouple. The calorimeter was surrounded by a constant temperature bath (ice, liquid oxygen, or solid CO_2) and the rates of heating and of cooling were measured by the thermocouple.

¹ Original thesis submitted July, 1935. Doctoral thesis number 332.

An adiabatic method was used for the runs at room temperature and above. A water bath surrounding the calorimeter was kept at the same temperature as the sample by using a differential thermocouple. The temperature was measured by a thermometer.

CALCULATIONS AND RESULTS

In the adiabatic runs, all the electrical energy was used in heating the sample. The heat capacity of a slug, C_p , was calculated from the rate of rise of temperature, dT/dt , and the energy input in calories per minute.

$$C_p = \frac{(I^2 R) (60)}{4.1826} \frac{dt}{dT}$$

dt/dT was found by measuring the slope of the curve obtained by plotting time in minutes against temperature.

In the method in which the calorimeter was surrounded by a constant temperature bath, part of the electrical energy was used in heating the sample and part was lost to the surroundings. The heat capacity was calculated by the equation,

$$C_p = \frac{(I^2 R) (60)}{(4.1826) \left(\frac{dE}{dt} + \frac{dE'}{dt} \right) \left(\frac{dT}{dE} \right)}$$

dE/dt is the increase per minute of the e. m. f. of the thermocouple, dE'/dt is the similar rate of decrease on cooling, and dE/dT is the rate of change of temperature with respect to e. m. f. of the thermocouple as given by the calibration curve for the thermocouple.

The specific heat was calculated for each slug. The specific heat of Fe_3C was calculated by plotting specific heat against percentage of carbon and extrapolating to pure Fe_3C (6.6 per cent C). The following table gives specific heat and molal heat capacity at several temperatures. These are somewhat lower than the corresponding values obtained by Naeser.

Absolute temperature	Specific heat	Molal heat capacity
323.2	0.1358	24.38
303.2	0.1341	24.06
280.7	0.1301	23.34
225.8	0.1160	20.83
201.3	0.1087	19.51
161.2	0.0884	15.87
120.5	0.0694	12.46
102.4	0.0566	10.16

The entropy of iron carbide was obtained by plotting absolute temperature against molal heat capacity divided by temperature and integrating graphically by finding the area under the curve. The heat capacity values used for temperatures below 100° K. were obtained by comparing

the temperature-specific heat curve for Fe_3C to that for iron. Since the two are quite similar in the range investigated experimentally and approach more closely at low temperatures, it was assumed that near absolute zero the form of the curve for Fe_3C would be very much like that for iron. The entropy of Fe_3C at 25°C . was found to be 23.55 units.

For the formation of one mole of Fe_3C from its elements at 25°C .,

$$\Delta S = 23.55 - (3 \times 6.60 + 1.39) = 2.36 \text{ units.}$$

$$\Delta F = 12300 - (298.16)(2.36) = 11596 \text{ cal.}$$

This indicates that Fe_3C is unstable or metastable at room temperature.

SUMMARY

1. The heat capacity of iron carbide in steel has been determined from 102°K . to 323°K . (see table).
2. The entropy of Fe_3C at 25°C . is found to be 23.55 units.
3. The free energy increase accompanying the formation of one mole of Fe_3C from its elements at 25°C . is calculated to be 11596 cal.

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EFFICIENCIES OF PETROLEUM DISTILLATES AS COOLING MEDIA FOR INTERNAL COMBUSTION ENGINES¹

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Since various individuals have used petroleum distillates as cooling media for their automobile engines at various times and under various conditions, without knowing the effects of such cooling, it was considered logical, since the subject appears not to be recorded in the literature, to investigate the effects of petroleum distillate, such as kerosene or distillate, upon the motor and the cooling system.

Six different kerosenes were obtained on the market and were compared with radiator alcohol, radiator glycerine and radiator glycol. The distillation range of these materials is presented in table 1.

At 0° C., the viscosity of kerosene (38 seconds) is only slightly greater than that of water (34 seconds) but is less than that of a 50-50 mixture of radiator alcohol (51 seconds), radiator glycol (56 seconds) or radiator glycerine (58 seconds). At - 32° C., the kerosene has definitely lower viscosity (58 seconds) than the mixtures of glycol (168 seconds), alcohol (363 seconds) or glycerine (which froze at - 27° C.).

The flash and fire points of the various kerosenes (150° F. and 170° F., respectively, in the open cup) were definitely higher than those of a 50-50 alcohol mixture (66° F. and 78° F., respectively).

Corrosion tests were run on both tinned copper and aluminum strips with the result that the kerosenes are no more corrosive than good tap water, and far less corrosive than radiator alcohol.

Rubber radiator hose, both a poor (black) and a good (red) grade, was subjected to the action of the various solutions at high and low temperatures. These results are presented in tables 2 and 3. The significant difference appeared in the bursting strength. The black hose was badly decayed and broke at 25 per cent normal bursting pressure, whereas the red hose remained firm and broke at 65 per cent normal bursting pressure.

Motor tests were made to determine the rate of heating of the motor when using water, alcohol, kerosene and glycol. The kerosene caused the motor to heat quicker, but when equilibrium was reached, the kerosene caused only a slight increase in operating temperature. Road tests also showed the same results.

The following conclusions are drawn from the data:

1. Kerosene is less viscous at low temperatures than any of the materials commonly used as cooling media.
2. Kerosene is less dangerous as a fire hazard than a 50 per cent alcohol solution.
3. Kerosene offers no corrosion problem when in contact with metal surfaces.

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TABLE 1. *Distillation range (°F.) of anti-freeze solutions*

	Kerosenes						Radiator		
	A	B	C	D	E	F	Alco- hol	Glycerine	Gly- col
Initial	330	352	348	346	350	369	168	219	323
10%	392	410	382	391	382	401	170	219	368
20%	404	422	393	404	392	414	170	219	374
30%	414	432	404	414	400	420	170	219	374
40%	422	439	412	422	408	426	170	219	374
50%	429	446	420	432	416	432	170	288	374
60%	436	453	430	441	424	438	170	492	375
70%	443	461	441	450	434	444	171	522	376
80%	453	470	454	460	444	454	171	538	376
90%	468	485	470	476	469	468	172	620	378
End	485	514	494	485	504	499	368	Decomp.	410
Recovery	98.0	98.5	98.6	98.9	98.0	98.0	98.0	98.0	96.5
Residue	1.5	1.5	1.4	0.2	1.6	1.2	0.9	1.3
Loss	0.5	0.0	0.0	0.0	0.4	0.8	1.1	2.2

TABLE 2. *Effect of various solutions on black radiator hose (48 days)*

Hose lbs.	Solution	Temp. °F.	Burst- ing pres- sure lbs./in. ²	Increase in		Nature of failure
				Wt. Pctg.	Vol. Pctg.	
15	Kerosene	170	30	22.5	50.6	Inside badly decayed, leak occurred, through fabric; could not burst it.
9	"	170	60	23.6	47.1	Inside partially decayed; break normal short spiral split.
13	"	115	55	19.6	37.9	Rubber firm, break normal short spiral split of outer fabric followed by tearing of rubber.
2	"	115	75	24.0	46.5	
17	Glycol	170	105	3.6	3.6	
10	"	170	95	3.7	5.3	
5	"	115	90	1.5	5.7	
4	"	115	105	4.2	6.8	
11	Water	170	140	5.1	7.2	
19	"	170	90	5.2	7.1	
8	"	115	110	1.9	3.3	
20	"	115	135	1.8	2.1	
6	Glycerine	170	140	1.2	3.6	
16	"	170	125	1.3	2.1	
3	"	115	125	0.3	2.1	
12	"	115	145	1.0	2.8	
7	Alcohol	170	110	0.8	5.7	
18	"	170	110	4.3	6.5	
1	"	115	145	3.3	4.2	
14	"	115	135	2.8	3.6	
Original	125	

4. Kerosene is more severe than other cooling media on rubber hose, but a quality product stands up both in laboratory tests and under actual conditions.

5. Kerosene causes a motor to heat up slightly faster than the other solutions and attains a temperature of 15° F. higher under no load.

6. Kerosene can be used satisfactorily as a cooling medium in actual operation, even in quite warm weather, causing the motor to run about 30° F. hotter than with water.

TABLE 3. *Effect of various solutions on red radiator hose (48 days)*

Hose lbs.	Solution	Temp. °F.	Burst- ing pres- sure lbs./in. ²	Increase in		Nature of failure
				Wt. Pctg.	Vol. Pctg.	
17	Kerosene	170	105	33.1	54.6	In all cases the rupture was a short spiral split of the outer fabric, followed by tearing of the rubber.
19	"	170	150	29.9	49.2	
18	"	120	240	6.3	7.5	
20	"	120	230	4.3	7.1	
16	Glycol	170	195	4.4	3.5	
10	"	170	240	3.6	4.7	
13	"	120	250	0.6	0.0	
8	"	120	250	3.5	1.3	
11	Water	170	250	7.3	8.4	
2	"	170	245	8.9	10.0	
5	"	120	250	1.6	0.0	
15	"	120	230	1.7	0.0	
4	Glycerine	170	300	4.5	3.9	
12	"	170	255	1.7	0.0	
9	"	120	260	0.3	0.0	
6	"	120	300	0.9	0.0	
3	Alcohol	170	270	2.4	0.7	End swelling due to outside leak.
1	"	170	250	1.5	0.7	
7	"	120	250	15.8	14.8	
14	"	120	260	3.3	0.7	
Original	200	

THE EFFECT OF HIGH FREQUENCY EXCITATION UPON THE INTENSITIES OF SPECTRAL LINES¹

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High frequency electric discharges in hydrogen were excited by voltages applied to a discharge tube by means of external electrodes. The spectrum of the discharge was studied as a function of the applied frequency. In particular the relative intensities of the Balmer series were measured for a series of frequencies and pressures.

The source of high frequency potential was that developed across the plate tank circuit of a push-pull oscillator which used No. 852-type tubes. The radio-frequency voltage was applied to the discharge tube by means of sleeve electrodes of fine wire. The applied potential difference was calculated from known values of current, capacity, and frequency of oscillation in the tank circuit. A constant peak potential of 1600 volts was maintained at the electrodes which were spaced 30 cm. apart. Excitation wavelengths of from 5.1 to 32.5 meters were used.

The gas system was designed for maximum purity. The hydrogen, generated electrolytically, was dried in a P_2O_5 trap and admitted to a chamber containing a palladium diffusion tube. Passage of the gas into the discharge tube was controlled by varying the temperature of the palladium tube, this being accomplished by passing an electric current through it. Diffusion pumps using Apiezon oil "B" were used and a Pirani gauge previously calibrated for hydrogen was used for pressure measurements. The whole vacuum system was of Pyrex glass except for a Corex window sealed in the end of the discharge tube. All joints up to the stop-cock at the pumps were glass sealed. Impurities due to wax and grease were thus eliminated and thorough baking of the entire tube at 500°. C. was possible. A trap between the discharge tube and pumps was cooled with solid CO_2 and acetone.

The discharge tube was 4.8 cm. in diameter and 75 cm. long. This length was greater than the theoretical minimum length for the frequencies and pressures used as calculated from J. Thomson's² equation for the initiation potential for this type of gaseous discharge.

$$E > \phi(f) \left[\frac{V_p}{K} + \frac{4\pi^2 f^2 K}{2p e/m} \right]$$

where E represents the electric field, V the ionization potential of the gas, K — the mean free path of an electron, f the frequency of oscillation, and p

¹ Original thesis submitted July, 1935. Doctoral thesis number 331.

² Thomson, J. 1930. On the mechanism of the electrodeless discharge. *Phil. Mag.* (7). 10: 280-291.

e/m the ratio of charge to mass for the electron, $\phi(f)$ is a function which represents loss of ions due to diffusion and which probably varies inversely with the square of the frequency of excitation. This function would also vary with different geometrical conditions such as tube dimensions.

The range of pressures studied in this investigation was from 300 to 4 microns. Beyond these limits it was impossible to obtain data for all the desired excitation wavelengths and maintain a constant potential at the electrodes.

Intensity ratios were obtained from spectrograms taken in conjunction with a logarithmic sector disc which gives spectral lines whose differences in length are proportional to their intensity ratios. Line lengths were measured to 0.1 mm. consistently, this corresponding to an accuracy of not more than 10 per cent error in the value of an intensity ratio. Spectrograms were taken with a Bausch and Lomb medium quartz spectrograph. W. and W. panchromatic plates of the same emulsion batch were used and were developed with developer formula D-19 from the same stock solution under the same conditions of temperature and time.

Considerable difficulty was encountered in attempting to rid the discharge of all spectroscopic traces of impurities. The most persistent of these appeared in the ultra-violet region and were the 3085Å water-vapor band and the 2882Å and 2899Å CO₂ bands, the latter appearing only at pressures of a few microns or less. Baking of the glass tube had no effect upon the appearance of these impurities. The water-vapor band could be eliminated by baking and pumping only if no hydrogen were present. It is believed that it results from the combination of hydrogen with oxygen released from the SiO₂ of the glass walls by electron bombardment. The CO₂ appearing at low pressures perhaps also results from some similar action.

A series of spectrograms of the discharge was obtained for a range of frequencies between 5.1 and 32.5 meters, while the pressure and applied potential difference were held constant. Data for six pressures of from 5 to 300 microns were obtained.

The results of the investigation show that:

1. There is a distinct variation in intensity ratios with excitation frequency.

2. There is a region of excitation frequencies which gives a minimum excitation of each term with respect to H α and that this region of frequencies depends upon pressure and the term under consideration.

3. Ionization efficiency and intensities of the higher terms of the series vary (more or less directly) with each other if cumulative ionization is negligible.

4. Intensities of the higher terms of the Balmer series increase as the pressure decreases to about 10 microns. For further diminishing of pressure H β and H γ continue to increase in intensity while the higher terms decrease.

5. Population of the energy states giving H β and H γ increase with decreasing pressure but vary relatively with the frequency of excitation voltage.

6. A value, $e^{-1} \left(\frac{f}{10^7} \right)^2$, can be chosen empirically for $\phi(f)$ of J.

Thomson's equation for the initiating potentials for this type of discharge. With this function the equation yields curves which are in good general agreement with the experimental results.

7. Some gas impurities appearing in the ultra-violet and not apparent in the visible region are inherent in a hydrogen discharge excited by high frequency potential and are probably due to electronic bombardment of the glass.

THE DIELECTRIC CONSTANT AND THE SPECIFIC CONDUCTANCE OF PURE LIQUID HYDROGEN SULPHIDE¹

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I. THE PRODUCTION OF PURE HYDROGEN SULPHIDE

The hydrogen sulphide gas was produced by the method of Habermann (1). This method utilizes the reaction between solid calcium sulphide and a saturated solution of magnesium chloride. Randall and Bichowsky (2) report that "the gas (hydrogen sulphide) is probably purer than that prepared by any other known method." The hydrogen sulphide gas was washed with water, barium sulphohydrate suspension, dried with calcium chloride, aluminum sulphide, and phosphorus pentoxide. It was then passed through a baffle for the removal of entrained solids, liquefied at atmospheric pressure by means of a dry ice-ether mixture, and finally fractionally distilled into the test cell.

II. THE A. C. BRIDGE METHOD

The conventional Kohlrausch method for determining electrical conductance employs a parallel arrangement of capacitance and resistance for balancing the impedance of the filled test cell. Difficulties are introduced into this method when the balancing resistance becomes quite large (500,000 ohms and larger) because of the impossibility of producing wire wound resistors of this magnitude that are reactance free. Since the specific resistivity of liquid hydrogen sulphide was known to be quite large (3) (4) (5) (6) it was found expedient to measure the power ratio, N_x (ratio of equivalent series resistance to reactance) of the liquid by means of Siskind's capacitance bridge (7) and to compute the equivalent parallel resistance, R_p , by the aid of the following equations:

$$N_x = R_x \overline{C_x} \quad (1)$$

$$R_x = N_x / \overline{C_x} \quad (2)$$

$$R_p = 1 / \omega^2 \overline{C_x}^2 R_x \quad (3)$$

In the above equations

N_x = power ratio

R_x = equivalent series resistance

$\overline{C_x}$ = capacitance of filled test cell

ω = $2\pi \times$ frequency in c.p.s.

In regard to equation 3 see Hague (8), page 136.

¹ Original thesis submitted March, 1936. Doctor thesis number 357.

III. THE CELL CONSTANT

The following method was used in determining the cell constant of the test cell. It is proposed to avoid difficulties encountered in the evaluation of the cell constant for a cell of large plate area and of small plate separation.

The cell constant, k_c , of a cell whose plate separation is d and whose uniform cross section is A , is expressed by the following equation:

$$k_c = \frac{d}{A} \quad (4)$$

The capacitance in micro-micro farads of a parallel plate condenser (when air filled) whose plate separation is d , and whose uniform cross section is A is expressed by

$$C_{\mu\mu_F} = \frac{1}{(.9)4} \times \frac{A}{d\pi} \quad (5)$$

Solving equation 5 for d/A it is found that the cell constant, k_c , is

$$k_c = \frac{d}{A} = \frac{0.08841}{C_{\mu\mu_F}} \quad (6)$$

This derivation was extended to the case of a cell having coaxial cylinders for electrodes with the result that

$$k_c^0 = \frac{0.08841}{C_{\mu\mu_F}} \quad (7)$$

This equation (7) holds rigorously when the distance between the electrodes is small compared to the diameter of the cylinder.

IV. THE SCREENED AUDIO-FREQUENCY CAPACITANCE BRIDGE

Siskind's (7) network was employed. The component parts were contained in individual compartments of an earthed copper alloy box. The oscillator and detector were contained in individual earthed copper alloy shields, and were connected to the bridge proper by means of duplex cables in earthed shields.

1. *The oscillator.* A vacuum tube generator similar in design to that of Shedlovsky (9) was used.

2. *The detector.* Western Electric headphones and a two-tube resistance coupled amplifier were employed in the detecting device.

3. *The Wagner-Ground circuit.* This unit was composed of two Ayrton-Perry coils, a ten ohm rheostat, and two $250\mu\mu_F$ condensers.

4. *The ratio arms.* This unit was a standard part from the General Radio type 216 capacity bridge (10).

5. *Balancing condensers.* These condensers were General Radio products with the exception of one Leeds and Northrup standard con-

denser. The General Radio Precision Condenser used for the determination of capacitances of the test specimens was calibrated so that a capacitance difference of $0.2\mu\mu_F$ or 0.1 per cent (whichever was the larger) could be detected.

V. THE TEST CELL

The test cell was essentially the same as that described by Field (11). It was constructed of nickel electrodes provided with guard rings. The important feature in the design of this cell is the absence of solid supports between the high and low potential electrodes. Such a cell as this exerts no shunting effect upon the liquid being measured. The "direct capacitance" of this cell was $40.40\mu\mu_F$. After substituting this value in Equation 7, the cell constant was found to be $2.18_8 \times 10^{-8}$.

VI. THE ELECTRICAL CONSTANTS FOR PURE LIQUID HYDROGEN SULPHIDE

The dielectric constant of a liquid is found by the ratio of the "filled" to the "empty" capacitance of the test cell.

The following values were found for the constants of hydrogen sulphide at a temperature of -78.5°C .

Dielectric constant— 9.0_5

Specific conductance— $3.1 \times 10^{-10} \text{ohm}^{-1} \text{cm.}^8$

The following values have been reported previously.

Specific conductance

$$0.1 \times 10^{-8} \text{ ohm}^{-1} \text{ cm.}^8 \quad (3)$$

$$\text{less than } 4.0 \times 10^{-7} \quad " \quad " \quad (4)$$

$$3.7 \times 10^{-11} \quad " \quad " \quad (5)$$

$$1.0 \times 10^{-11} \quad " \quad " \quad (5)$$

Dielectric constant

$$10.2 \quad (4)$$

$$9.4 \quad (12)$$

Probably the best previous measurements on the specific conductance were made by Wilkinson and co-workers (5) (6) in this laboratory. Their reported values are somewhat less than the present observation. This is to be expected in view of the fact that they employed a direct current galvanometer method and encountered some polarization. Taking this into account the agreement between the new and old data from this laboratory is good.

Kemp and Dennison (12) observed the dielectric constant at the melting point (-82.5°C .) while the present observation is made at -78.5°C ., which perhaps accounts for the difference, although Kemp and Dennison claim only a precision of ten per cent. Obviously the value of Magri (4) is widely in error.

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PHYSIOLOGICAL STUDIES AND CLASSIFICATION OF THE BUTYRIC ACID-BUTYL ALCOHOL BACTERIA¹

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Morphological, cultural and physiological characteristics of 36 strains of butyric acid-butyl alcohol bacteria were investigated. The following seven species were differentiated. *Clostridium amylobacter*, *Cl. acetobutylicum*, *Cl. felsineum*, *Cl. butyricum*, *Cl. saccharobutyricum*, *Cl. Pasteurianum* and *Cl. Beijerinckii*.

All the species are anaerobic, sporulating, rod-shaped, gram positive and catalase negative. Granulose is present during the clostridial stage. Nitrates are not reduced and indol is not formed. H₂S is produced from thiosulphates. The characteristic products of dissimilation are butyric and acetic acids, butyl alcohol, CO₂ and H₂. Glucose, sucrose, lactose, maltose, xylose, levulose, mannose, galactose, amygdalin, salicin and cellobiose are fermented. Adonitol, dulcitol and erythritol are not attacked.

Acetylmethylcarbinol is a normal product of dissimilation by *Cl. acetobutylicum* and *Cl. felsineum* but 2,3-buteneglycol is not produced. In the presence of peptone and glucose the carbinol is not reduced.

Acetomethylcarbinol does not occur as an end product of dissimilation by *Cl. butyricum*, *Cl. amylobacter*, *Cl. saccharobutyricum*, *Cl. Pasteurianum* and *Cl. Beijerinckii*. 2,3-Butyleneglycol is normally produced. Added carbinol is completely reduced to the glycol.

The fermentation of 5 per cent corn mash by *Cl. felsineum* and *Cl. acetobutylicum* was characterized by extensive hydrolysis of proteins and starch, with the formation of high yields of butyl alcohol and acetone. Of the weight of corn fermented, 20 to 30 per cent was converted to neutral volatile products. The remaining species were relatively non-proteolytic. Corn mash was only partially fermented with the formation of mostly acid products.

Tatum, Peterson and Fred (1934, 1935) reported that asparagin stimulated the production of butyl alcohol from the fermentation of corn mash by butyric acid anaerobes. No increase in other neutral products was observed.

In the investigation reported here, it was found that only *Cl. butyricum* was stimulated by the addition of asparagin to corn mash. Moreover, in the presence of asparagin, peptone, yeast extract and mixtures of amino acids, corn mash was converted into as much as 17 per cent butyl and 9 per cent isopropyl alcohols. The addition of these nitrogenous substances to corn mash was without effect on the yields of "solvents" by the other species. Although *Cl. butyricum* was unable to utilize undegraded proteins as source of nitrogen, no specific amino acids appeared to be essential to the metabolism of this species.

¹ Original thesis submitted June, 1936. Doctoral thesis number 366.

Pyruvic acid, which has been suggested by several investigators as an intermediate product in the butyric acid-butyl alcohol fermentation, was converted by cell suspensions of *Cl. butyricum* into acetic and butyric acids, CO_2 and H_2 . Hydrogen, donated by pyruvic acid, was available for the reduction of butyric acid and acetone to butyl and isopropyl alcohols respectively. Lactic and formic acids were not attacked by cell suspensions of this species.

The following key for the differentiation of the species was proposed:

A. Bacteria producing considerable amounts of butyl alcohol, acetone and ethyl alcohol from corn without added nitrogenous substances. Strongly proteolytic. Gelatin liquefied. Pink or orange-yellow pigment produced in corn mash. Melibiose and trehalose not attacked. 2,3-Butyleneglycol not formed. Voges-Proskauer positive.

B. Not fermenting raffinose, rhamnose, inulin and pectin. Fermenting mannitol and α -methylglucoside. Orange-yellow pigment in corn mash.

Clostridium acetobutylicum.

BB. Fermenting raffinose, rhamnose, inulin and pectin. Not fermenting mannitol and α -methylglucoside. Pink pigment in corn mash.

Clostridium felsineum.

AA. Producing considerable amounts of butyl and isopropyl alcohols from corn when an available source of nitrogen is present. Non-proteolytic. Gelatin not liquefied. Voges-Proskauer negative. 2,3-Butyleneglycol produced. No pigment produced. Melibiose, trehalose and rhamnose fermented.

Clostridium butyricum.

AAA. Very small amounts of alcohols produced from corn with or without added nitrogenous substances. Non-proteolytic. Gelatin not liquefied. Rhamnose not fermented.

B. Fermenting starch, dextrin, glycogen and glycerol.

C. Fermenting melezitose, sorbitol, inulin, arabinose and mannitol. Not fermenting pectin.

Clostridium Pasteurianum.

CC. Not fermenting melezitose, sorbitol, inulin, arabinose and mannitol.

D. Not fermenting pectin.

Clostridium saccharobutyricum.

DD. Fermenting pectin.

Clostridium amylobacter.

BB. Not fermenting starch, dextrin, glycogen and glycerol.

Clostridium Beijerinckii.

In view of the probability that *Bacillus amylobacter* Van Tieghem (1877) was a pectin fermenting organism and since this specific name

should be recognized for the type species of the genus *Clostridium* Prazmowski (1880), it was proposed that the "retting organism" previously designated as *Plectridium pectinovorum* Stormer (1903), *Granulobacter pectinovorum* Beijerinck and Van Delden (1904) and *Cl. pectinovorum* Donker (1926), be named *Clostridium amylobacter* and be recognized as the type species. Moreover, it was proposed that the isopropyl alcohol producing species be designated as *Cl. butyricum* Prazmowski since it is highly probable that this specific name was originally assigned to an organism of that type.

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THE RESOLUTION OF ALPHA-SUBSTITUTED PYRROLIDINES¹

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Attempts to discover a quantitative connection between rotatory power and chemical constitution of an active molecule have been numerous since Crum Brown² and Guye³ published the theory connecting certain constants representing the radicals with molecular rotatory power. Their calculations of rotations from the radicals attached to the asymmetric carbon atom proved to be unsuccessful even for homologous series. Betti⁴ and Rule⁵ obtained very good correlations in some series of compounds when they considered the electrical properties of the radicals as measured by dissociation constants and dipole moments. Betti compared the rotations of aldehydo-aminic derivatives of β -naphtholphenylamino

methane $C_6H_5 - \overset{H}{\underset{|}{C}} - N = \overset{H}{\underset{|}{C}} - R$, with the dissociation constants of the $C_{10}H_5OH$

acids, $RCOOH$, where R is a substituted phenyl radical. Rule compared rotations of a series of homogeneous 1-menthyl esters of mono-substituted acetic acids, XCH_2-COOH , with the dissociation constants of the acids. According to these investigators increasing the "electronegativity" of one of the four radicals attached to the asymmetric carbon atom increased the molecular rotation. Levene⁶ found that in the derivatives of disubsti-

tuted acetic acids, $R_1 - \overset{H}{\underset{R_2}{\underset{|}{C}}} - COOH$, the direction of the rotation and the

respective numerical values of the latter, might be regarded as functions of the polarity of the substituting group. From these results it can be seen that the electrical properties of substituents exert a marked influence on the rotary power of optically active compounds.

To initiate a study of the effect on the rotatory power due to varying the radical, R, attached to the asymmetric carbon atom in a series of α -substituted pyrrolidines, the compounds were prepared and resolved. The preparations were accomplished according to a general method developed by Craig, Bulbrook and Hixon⁷. α ,p-Chlorophenylpyrrolidine and the corresponding pyrroline were prepared for the first time; α ,n-butylpyrrolidine and the corresponding pyrroline were prepared by this method

¹ Original thesis submitted December, 1935. Doctoral thesis number 350.

² Crum Brown, *Proc. Roy. Soc., Edinburgh*, **17**, 181. 1890.

³ Guye, *Compt. rend.*, **110**, 714. 1890.

⁴ Betti, *Gazz. chim. ital.*, **50**, II, 276. 1920.

⁵ Rule, *J. Chem. Soc.*, **125**, 1121. 1924.

⁶ Levene, *J. Biol. Chem.*, **84**, 571. 1929.

⁷ Craig, Bulbrook and Hixon, *J. Am. Chem. Soc.*, **53**, 1831. 1931.

for the first time. α -Phenylpyrrolidine, α -ethylpyrrolidine, α , p -tolylpyrrolidine and α -cyclohexylpyrrolidine were prepared. They were resolved for the first time into dextro and laevo forms. Dextro tartaric acid was the resolving agent for the d-base and laevo-tartaric acid for the l-base of the first three compounds mentioned. Dextro camphoric acid was used to obtain both the d- and l-forms of α -cyclohexylpyrrolidine. Attempts at resolving α , p -chlorophenylpyrrolidine, α , n -butylpyrrolidine and α -benzylpyrrolidine were unsuccessful. Micro Dumas nitrogen analyses were made on all new compounds, and others were characterized by boiling points or melting points and derivatives. The densities and refractive indices of the bases were determined. Rotations of the pure pyrrolidines and of ethyl alcohol solutions of the pyrrolidines were measured with a sodium arc as the source of light. Rotations of alcohol and of water solutions of the salts of the active bases were read.

If comparisons of rotatory powers are to be made it is necessary to compare values for active forms having the same configuration. d,α -Phenylpyrrolidine was reduced by hydrogenation under pressure in the presence of platinum oxide platinum black catalyst. The product obtained from this reduction was proved to be identical with the d,α -cyclohexylpyrrolidine obtained from the resolution of the racemic mixture with d-camphoric acid. Thus d,α -cyclohexylpyrrolidine and d,α -phenylpyrrolidine have the same configuration. It is assumed that the dextro forms of α -ethylpyrrolidine and α -cyclohexylpyrrolidine have the same configuration as the dextro forms of α -phenylpyrrolidine and α -cyclohexylpyrrolidine.

When the radical, R, is varied the molecular rotation of the liquid active α -substituted pyrrolidines varies. Changing R from a relatively "electropositive" group, cyclohexyl, to a relatively "electronegative" group, phenyl, changes the rotatory power from +14.38 at 25° to +103 at 25°. The other two members of the series show molecular rotations between these two values.

The influence exerted by R on the molecular rotatory power is compared with the effect on the dissociation constants and with similar variations in a series of α -substituted ethylamines. When the dissociation constants of these compounds in methanol and in water and the dissociation constants of the corresponding primary amines, $R-NH_2$, are arranged in decreasing order of magnitude, the rotatory powers of the α -ethylamines and α -substituted pyrrolidines with the exception of the α -ethylpyrrolidine, are found to fall in increasing order of magnitude. An increase in the dissociation constants of the corresponding acids, $R-COOH$, corresponded to an increase in the molecular rotations of the active compounds.

Burch⁸ determined the values for the dissociation constants of the α -ethylamines in methanol which are nearly equal to the values obtained for the α -substituted pyrrolidines. In comparing the rotatory powers of the two series a large difference in the values for the molecular rotations having like R substituents is observed. The presence of the ring in the α -substituted pyrrolidines seems to influence the rotary power much more than it does the dissociation constant. The configurations in the two

⁸ Burch, unpublished thesis for Ph. D. Iowa State College Library. 1935.

series are also dissimilar. In the α -substituted ethylamine series Leithe⁹ converted α -phenylethylamine into α -cyclohexylethylamine by catalytic hydrogenation and showed that they had opposite signs of rotation for the same configuration. The dextro form of α -phenylpyrrolidine was reduced to the dextro form of α -cyclohexylpyrrolidine showing that the sign of rotation remains the same.

It is concluded that some relationship exists between the optical rotatory power of the α -substituted pyrrolidines and the electrical property of the radical, R, as measured by dissociation constants and that the relation is even more complex than the one exhibited by the α -ethylamines.

⁹ Leithe, *Ber.*, 63, 800. 1930.

A STUDY OF 2,3 BUTYLENE GLYCOL AND ITS DERIVATIVES¹

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Recognition of their usefulness and extreme versatility has brought about an ever increasing production of organic compounds. New compounds are constantly being developed to meet specific needs and new uses are being discovered for products already known. The sources of raw materials for the synthesis of these compounds may be divided into two classes: the organic deposits in the earth, the amount of which, though large, is limited, and the practically inexhaustible supply of agricultural products. The gradual depletion of the first source will force a turn to the greater utilization of the latter. Because of this, and because a greater production and more varied use of agricultural produce will satisfy the long-felt economic need for more stable agricultural prices, it seems expedient to try to enlarge our knowledge of the fundamental compounds obtainable from plants, so that we can produce and apply them most efficiently. 2,3 Butylene glycol is such a compound and the object of this study was to add somewhat to the information concerning it.

Though 2,3 butylene glycol was first isolated from a fermentation mixture in 1906 (1), and small amounts had been synthesized by a variety of methods prior to that date, it was not until recently that much attention was given to its possibilities as a useful industrial chemical. However, the glycol is now being produced on a semi-commercial scale and is available at a moderate price. In these laboratories, in 1934, Kendall (2) developed the conditions for maximum conversion of sucrose to 2,3 butylene glycol by fermentation and this thesis is intended to supplement that work by presenting some of the properties of the glycol and its derivatives.

With the help of Kendall's findings, nearly 2600 g. of 2,3 butylene glycol were synthesized biochemically. Recovery from the beer was effected by means of continuous ether extraction. A diagram of the extraction apparatus and a brief discussion of the problems involved are given. The vapor pressure of the glycol was determined at several temperatures and shown to be fairly low, even at 100° C.

The preparation, in many instances by several methods, of the following derivatives of 2,3 butylene glycol is described, and the boiling-points, densities, and refractive indices of these compounds reported: 2,3 butylene bromohydrin, 2,3 butylene chlorohydrin, 2,3 butylene dibromide, 2,3 butylene dichloride, 2,3 butylene glycol monomethyl ether, 2,3 butylene glycol diethyl ether, 2,3 butylene glycol mono-isopropyl ether, 2,3 butylene glycol di-n-propyl ether, 2,3 butylene glycol mono-iso-propyl ether, 2,3 butylene glycol mono-n-butyl ether, 2, 3 butylene glycol di-n-butyl ether, 2 bromo 3 methoxy butane, 2 bromo 3 ethoxy butane, 2 chloro 2 methoxy butane,

¹ Original thesis submitted July, 1935. Doctoral thesis number 333.

2 chloro 3 ethoxy butane, 2,3 butylene glycol diacetate, 2,3 butylene glycol mono-methyl ether acetate, and 2,3 butylene diamine.

Because of its theoretical importance as a fundamental compound and the comparatively little information available concerning it, considerable attention is devoted to 2,3 butylene diamine. The substance was synthesized by many methods, including those of previous workers, and the conclusion drawn that reduction of dimethyl glyoxine in alcoholic hydrochloric acid, using platinum black as the catalyst, is the most expedient, though reduction of the glyoxine in acetic anhydride would be much better if the resulting acetyl derivative were more readily hydrolyzed. Gradual addition of hydrochloric acid during the reduction, instead of all at the beginning, was found to increase the yield. A diagram of a flask developed for these reductions, as well as graphs for the reductions in glacial acetic acid and in acetic anhydride, are shown.

An electrometric titration of the diamine was run and the dissociation constants calculated from the data obtained. They are (at 25° C.): $K_1 = 5.5 \times 10^{-5}$; $K_2 = 2.9 \times 10^{-8}$. The preparation and melting points of several derivatives characterizing the diamine are given.

The 2,3 butylene diamine prepared was resolved by means of *da*-bromocamphor π sulphonic acid. The salt of the dextro isomer was obtained quite pure, but that of the laevo form could not be crystallized. The values found for the optical rotation of the two forms were $[\alpha]^{25}_D = +7.3$ and -5.0° , the latter figure being for a 10 per cent aqueous solution. Strack and Schwaneberg (3) resolved the diamine just recently, using tartaric acid, and obtained a value of $[\alpha]^{18}_D = \pm 4.8^\circ$ for a 5 per cent aqueous solution. Dextro 2,3 butylene glycol, $[\alpha]^{25}_D = +6.9^\circ$ was prepared from the dextro diamine and was in turn converted to laevo-rotary 2,3 butylene dichloride $[\alpha]^{25}_D = -15.36^\circ$.

A discussion of the maximum possible yield of 2,3 butylene glycol biochemically obtainable from raw materials is presented, together with a proposed mechanism of reaction.

The properties of the derivatives of 2,3 butylene glycol fall midway between those of the simple monohydric alcohols and glycerol. They are very similar to the properties of ethylene glycol, but exhibit a little more of a hydrocarbon nature. Many of the butylene glycol derivatives are excellent solvents and might find application in cases in which their specific properties such as boiling-point, volatility, and viscosity, would make them more desirable than similar derivatives of glycerol or ethylene glycol. If the present day manifold uses of glycerol and ethylene glycol were examined, many instances undoubtedly would be found in which 2,3 butylene glycol would be superior, and many other cases in which the butylene glycol would be an admirable substitute if the price of glycerol or ethylene glycol should get out of bonds, as was true during the World War.

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THE ANIMAL PARASITES OF THE WOODCHUCK (*MARMOTA MONAX* L.) WITH SPECIAL REFERENCE TO PROTOZOA¹

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The present investigation was undertaken in order to secure more definite data on the morphology, life histories and other biological factors regarding the protozoa of the common woodchuck, *Marmota monax* Linn., and to make a complete record of all animal parasites known to live on or within that host species. The genus *Chilomastix* Alexeieff has been given special consideration among the protozoa, since many factors regarding morphology, method of division, and binuclearity in both trophozoites and cysts are not well understood. Further considerations have been given *Trichomonas digranula* Crouch and *T. wenrichi* Crouch. New species of *Chilomastix* and *Endamoeba* have been described.

MATERIALS AND TECHNIQUES

Parasites were collected from 56 woodchucks; 35 from the vicinity of Ames, Iowa, and 21 from Franklin County, Kentucky. Cover glass smears were made from the intestinal content fixed in Schaudinn's fluid, and stained according to Heidenhain's iron-alum hematoxylin method. Delafield's hematoxylin was also used, but did not give very good results.

THE PROTOZOAN PARASITES

Chilomastix instabilis, n. sp., is morphologically similar to other species of the genus. There are four or six blepharoplasts anterior to the nucleus, to which the three anterior flagella, cytostomal flagellum, parabasal body, and parastyle are attached. These blepharoplasts are connected by short fibrils. Becker's parabasal body is found in the majority of specimens, but it is not known whether it is attached to blepharoplasts or to other structures. The cytostomal flagellum is not attached to an undulating membrane. The average size of this species is $12.1\mu \times 9.45\mu$.

The cysts of *C. instabilis* are typically egg-shaped. The average size is $9.45\mu \times 8.19\mu$. No intracystic mitosis or binucleate cysts have been observed. Binucleate trophozoites are fairly common, and it is probable that binucleate cysts result from encystment of these forms.

The process of division in *C. instabilis* is different in some respects from other species. The blepharoplasts do not enter in the formation of the mitotic spindle during division. They remain in their normal positions at the anterior end of the body and ultimately degenerate. A centrosome, situated on or near the anterior margin of the nucleus, becomes the division center. None of the organelles associated with the blepharoplasts migrate with the nucleus during division. New sets of organelles are produced from the young blepharoplasts at the ends of the mitotic spindle.

¹ Original thesis submitted June, 1936. Doctoral thesis number 368.

Approximately eight chromosomes appear during the early stages of division.

The check list of the genus *Chilomastix* includes 24 names of species exclusive of *C. instabilis*, and 26 records in which the species was not designated. The total number of species is reduced to 16 through synonymy and homonymy. Fifty-nine host records are listed.

Endamoeba marmotae n. sp., is similar to other *E. coli*-type *endamoebae*. The average size of mononucleate trophozoites is $16.56\mu \times 14.11\mu$; trophozoites with more than one nucleus are usually larger. The endoplasm and ectoplasm are poorly differentiated. The pseudopodia are finely granulated. The cysts measure 12μ in diameter, on an average. The cyst wall is about 0.5μ thick. Eight nuclei occur in the fully mature cysts. The chromatoid bodies are rather large and rod-shaped.

Trichomonas digranula appears to be a primitive member of the genus. The "adult" of this species is similar to early division stages in other species. The connecting rhizoplast between the blepharoplasts becomes functional as the paradesmose during division.

Binucleate trophozoites of *T. digranula* have a striking similarity to some members of the genus *Hexamita*. It is suggested that both the highly developed members of the genus *Trichomonas* and the hexamitids probably arose from a primitive ancestral trichomonad similar to *T. digranula*.

Trichomonas wenrichi has a life history similar to most other reported species. A new chromatic basal rod grows from the blepharoplasts before any other apparent changes occur. The prophase chromosomes are constricted in the middle, and appear to be composed of two nearly spherical masses. There are six chromosomes in this species. The karyosome is visible only during the organization of the prophase chromosomes. The parent axostyle does not completely degenerate during division. From the fact that one of the daughter axostyles is usually larger than the other, it seems probable that the old axostyle contributes in some way to the production of the larger daughter axostyle. The parent chromatic basal rod is retained in one of the daughter trophozoites.

Somatomy, or fragmentation of the cytoplasm, is noted in *T. wenrichi* and other trichomonads. Globules of cytoplasm are pinched off from the posterior end of the body. Some of these globules contain undigested materials and *Sphaerita* parasites, while others contain no solid matter. Somatomy appears to serve as a method of eliminating waste materials. It also serves as a method of overcoming hyperparasitism. From the fact that metabolic products are probably eliminated in this manner, somatomy may function as rejuvenation process.

HYPERPARASITISM

Sphaerita parasites are found in all species of protozoa from the common woodchuck, with the exception of the coccidia and *Hexamita*. At least two species of *Sphaerita* are present. These hyperparasites do not appear to be highly pathogenic to their parasite host.

The following species of protozoa are recorded from the common woodchuck, *Marmota monax*: MASTIGOPHORA—*Chilomastix instabilis*, n. sp., *Trichomonas cryptonucleata* Crouch, *T. digranula* Crouch, *T. marmotae* Crouch, *T. wenrichi* Crouch, *Hexamita marmotae* Crouch; RHIZOPODA—*Endamoeba marmotae* n. sp.; SPOROZOA—*Eimeria*

monacis Fish, *E. os* Crouch and Becker, *E. perforoides* Crouch and Becker.

PARASITES EXCLUSIVE OF THE PROTOZOA

NEMATHELMINTHES: *Ascaris laevis* Leidy, *Citellina marmotae* Manter, *Citellinema monacis* Manter. ARTHROPODA: Insecta-Cyclophthirus (= *Enderleinellus*) *marmotae* (Ferris), *Oropsylla arctomys* (Baker), *Opisodasys pseudoarctomys* (Baker), *Orchopeas wickhami* (Baker), *Ctenocephalides canis* (Curtis), *Ixodes hexagonus* Pack., *Dermacentor variabilis*, *Atricholaelaps glasowi* (Ewing), *Ichoronyssus sternalis* Ewing, *Trombicula blarinae* Ewing.

A TOXICOLOGICAL INVESTIGATION OF NICOTINE ON THE GOLDFISH AND THE COCKROACH

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PART I

THE EFFECT OF SOLUTIONS OF THE BASE AND SULPHATE ON THE GOLDFISH, *CARASSIUS AURATUS* (L.)¹

The difference in the physiological action exhibited by the molecules and the ions of various alkaloids, when used as anesthetics and insecticides, has attracted the attention of a number of research workers. Most of the entomologists who first investigated nicotine believed that the greater toxicity of the undissociated molecule resulted from its greater volatility. This theory, however, did not fully explain the difference in toxicity, for it was shown that there was still a marked difference when certain experimental animals were exposed to solutions containing largely nicotine molecules and others to solutions largely of dissociated nicotine.

According to the results obtained by other investigators, the free base of most alkaloids exhibits a more marked toxic action, when applied to the external surface, than solutions of the salt of the same molar concentration. However, it appears that conclusive evidence has not been presented which fully explains the cause of this phenomenon.

It was thought that additional light could be thrown upon the subject by approaching the matter from a quantitative basis. Consequently, quantitative determinations were made of the amount of nicotine in the goldfish, *Carassius auratus*, after death in solutions of the free base and in solutions of the sulphate. In addition, quantitative determinations were made of the speeds of penetration of nicotine base and of its sulphate into the body. The quantity of nicotine that the goldfish could tolerate without lethal effects, and the distribution of nicotine (from solutions of the base and sulphate) in the various tissues of the organism were also investigated.

A review of the literature was made, and 33 of the more pertinent references were discussed in the thesis. The source and care of the biological material and the chemical composition of the materials used in the investigation were given. Also, the method of handling the goldfish, exposing them to the test solutions, extracting the nicotine from the tissues and recovering it, and determining the quantity of nicotine in the test animals were discussed in detail. In addition, the criterion of toxic effect, the endpoint, was given.

In the first tests, the goldfish were exposed until death in solutions of nicotine base and of the sulphate of various pH values at concentrations of 0.002 M, 0.001 M, and 0.0002 M. At the time of death of the animals, the

¹ Original thesis submitted December, 1935. Doctoral thesis number 353.

quantity of nicotine recovered was approximately the same regardless of the pH of the solution in which they were exposed. For example, a mean of 0.034 mg./g. (milligram per gram of body weight) of nicotine was present in the bodies of the fish killed in solutions of 0.001 M nicotine base; whereas those killed in solutions of the sulphate at pH values of 7.3 and 5.0 showed a mean of 0.033 mg./g. and 0.034 mg./g., respectively. In solutions of the same molar concentration of nicotine, the free base penetrated much more rapidly than the partly ionized or completely ionized nicotine sulphate. In nicotine solutions of 0.002 M, the mean time to death was 102 seconds for the free base and 174 seconds, 355 seconds, and 1,141 seconds for the nicotine sulphate at pH values of 7.5, 5.0, and 2.6, respectively. Therefore, it is evident that the rate of entrance of the nicotine, hence the speed of toxic action, decreases with the increase in the ionization of the nicotine molecules.

The nicotine is not entirely absorbed in the skin, but a considerable quantity penetrates the deeper lying tissues. This was demonstrated by analysis of muscle tissue, integument, fins and gills taken from fish exposed to solutions of nicotine.

The survival time of fish when exposed to solutions of 0.002 M nicotine base was determined. It was found that 50 percent of the population failed to recover after being exposed for about 23 seconds.

Charts and tables of the experimental data are given in the thesis.

PART II

THE EFFECT BY INJECTION OF SOLUTIONS OF THE BASE AND SULPHATE INTO THE COCKROACH, *PERIPLANETA AMERICANA* (L.)

It was shown in the investigation on the goldfish that the greater toxicity of the free base of nicotine was a result of its more rapid speed of entrance into the organism. If the problem of penetration through the integument was eliminated by placing the toxic solution in direct contact with the blood, it seemed likely that the true relationship between the toxicity of the ions and of the molecules of nicotine could be demonstrated experimentally.

Accordingly, experiments were conducted in which nicotine solutions of known pH values were injected into the body cavity of the experimental animal, which was the American cockroach.

A review of the literature pertaining to injection of alkaloids into insects was made. The references are given in the thesis.

The apparatus for injection, and also the method of injecting the compound into the roach were described. Two criteria of toxic action were used in this investigation: one was death and the other was the period of time which elapsed from injection to complete paralysis and from injection to recovery from paralysis of the various pairs of legs.

In all cases 0.05 cc. of solution was injected, the temperature of which was 23° C.

In the first experiment molar concentrations of 0.05, 0.02, 0.013, and 0.01 nicotine base and sulphate were injected. The pH values of the sulphate were approximately 7 and 3. When death was the criterion of toxic action, it was found that at any given molarity of nicotine there were

no significant differences in the percentage of mortality of the roaches injected with the base and those injected with the sulphate.

Tests were also made with 0.01 M nicotine base and the sulphate at pH values of 6.7 and 2.8. In these tests, where the time from injection to paralysis and recovery of the pairs of legs was taken as the criterion of effect, again there were no significant differences in the toxicity of the free base and the sulphate.

These investigations on the goldfish and on the cockroach seem to point to the fact that the difference in toxicity of nicotine and nicotine sulphate is the result of the greater speed of penetration of the molecules. For, when the toxic effect is dependent upon the penetration of a membrane (body covering of the goldfish), the free nicotine is much more toxic than solutions of the sulphate of the same nicotine concentration; whereas, when solutions of either the ionized or molecular nicotine are injected directly into the cavity of the body (cockroach) there is no difference in toxic action.

Charts and tables of the experimental data are given in the thesis.

THE INFLUENCE OF VARIOUS PROCEDURES ON THE FLAVOR AND KEEPING QUALITY OF BUTTER¹

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Some of the constituents which are responsible for the desirable flavor and aroma of butter cultures have been studied in considerable detail. The work presented in this thesis was carried on in an attempt to determine the best methods of using culture in order to transfer its desirable qualities to the resulting butter.

The cream used was gathered cream of varying quality. In most of the comparisons sweet cream was employed, but in a few cases comparisons were made using sour cream.

The scores on the butter were treated statistically by the method suggested by Brandt (1) in order to determine whether the differences in score were highly significant, significant or not significant.

THE INFLUENCE OF THE METHOD OF USING BUTTER CULTURE ON THE FLAVOR AND KEEPING QUALITY OF BUTTER

The method of using butter culture was found to have considerable influence on the flavor and keeping quality of butter. The addition of 8 per cent butter culture to pasteurized and cooled cream 16 hours before churning resulted in butter significantly higher in score than the addition of 8 per cent culture at the time of churning; this was the case when the butter was fresh and also after cold storage, and occurred with both sweet and sour cream. Holding the pasteurized and cooled cream containing 8 per cent culture at a low temperature for 16 hours before churning had little effect on the acidity of the cream but did result in an increase in its acetylmethylcarbinol plus diacetyl content. Holding periods longer than 16 hours did not give appreciable improvements in the scores of the resulting butter. In some cases the acetylmethylcarbinol plus diacetyl content of the cream was increased during such holding periods but more often a decrease took place.

When 8 per cent butter culture was added to pasteurized cream that had been cooled to 70° F. and the cream ripened for 1 hour, the butter was more often high in score than the butter made by the addition of 8 per cent culture to pasteurized and cooled cream. The differences in score were not significant when the butter was fresh and after cold storage, but were significant after the butter had been held a few weeks at about 28° F. The ripening of the cream for 1 hour at 70° F. usually brought about an increase in the acetylmethylcarbinol content of the cream but not in the acidity.

Three methods of using culture gave butter which did not differ significantly in score when fresh, after holding a few weeks at 28° F. or after cold storage. These methods were: (A) the addition of 8 per cent

¹ Original thesis submitted June, 1936. Doctoral thesis number 382.

culture to pasteurized and cooled cream followed by holding at 28° to 36° F., (B) the addition of 8 per cent culture to pasteurized and cooled cream with holding at 41° to 52° F., and (C) the addition of 8 per cent culture to pasteurized cream at 70° F. followed by ripening for 1 hour, cooling and holding at 28° to 36° F. With methods A and B the acetylmethylcarbinol plus diacetyl contents were about equal and both methods resulted in higher acetylmethylcarbinol plus diacetyl contents than method C.

Butter made by the addition of 8 per cent culture to pasteurized and cooled cream was usually significantly higher in score than butter made without culture when scored fresh and also after holding a few weeks at about 28° F.; after cold storage there was commonly little difference in the scores. This was true with both sweet and sour cream. There was usually very little or no acetylmethylcarbinol plus diacetyl in the cream churned without culture.

THE INFLUENCE OF THE TYPE OF BUTTER CULTURE ON THE FLAVOR AND KEEPING QUALITY OF BUTTER

A modified culture was made as follows: *Streptococcus paracitrovorus* was grown in pasteurized milk at 70° F. for 24 hours; the milk was then acidified with 0.3 per cent sulfuric acid and 0.15 per cent citric acid, held another 24 hours at 70° F. and finally cooled to 40° F. With either sweet or sour cream, the use of this modified culture resulted in butter significantly higher in score than the use of regular culture, or the use of no culture, when the butter was scored fresh or after holding a few weeks at about 28° F.; after cold storage there was no significant difference in the scores.

Trials were made using regular and modified culture neutralized back to 0.3 per cent acid. The object in neutralizing the cultures was to attempt to retain the desirable flavors resulting from the use of culture without developing a sour flavor in the butter. The neutralized cultures did not give butter significantly higher in score than the unneutralized cultures when scored fresh, after holding a few weeks at about 28° F. or after cold storage. The use of either regular or modified culture that had been neutralized gave butter significantly higher in score than the use of no culture when the butter was fresh and also after holding a few weeks at about 28° F.; after cold storage there was not a significant difference in the scores.

It was noted that in some instances the desirable flavor imparted to butter by the use of butter culture disappeared on holding. Since butter cultures that are held for extended periods commonly show a decrease in the acetylmethylcarbinol plus diacetyl contents, as a result of the action of butter culture organisms, an attempt was made to prevent this destruction of the desirable flavor in butter by pasteurizing the ripened butter culture. The curd was filtered off and the filtrate added to the cream. However, butter made using regular culture was significantly higher in score than the butter made using pasteurized culture when scored fresh or after holding a few weeks at about 28° F.; after cold storage there was no significant difference in the scores.

THE INFLUENCE OF THE ADDITION OF ACETYLMETHYLCARBINOL AND DIACETYL TO BUTTER ON ITS FLAVOR AND KEEPING QUALITY

Butter made by the addition of diacetyl was not significantly different in score than butter made with butter culture when scored fresh, after holding a few weeks at about 28° F. or after cold storage. The addition of *Streptococcus lactis* or *Streptococcus paracitrovorus* to cream and acetylmethylcarbinol to the resulting butter did not give butter significantly different in score than butter made with butter culture when scored fresh, after holding a few weeks at about 28° F., or after cold storage.

THE MANUFACTURE OF HIGH SCORING BUTTER

The results indicate that in the manufacture of butter higher in score than any of the commercial grades the flavor of the cream and its subsequent treatment, with respect to neutralization and use of butter culture, are of extreme importance. The practices found of value were the addition of 0.15 per cent citric acid to milk used for butter culture, and the neutralization of sweet cream after pasteurization to 0.10 per cent acid before adding the culture.

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ON THE PENETRATION OF CERTAIN ARSENICAL COMPOUNDS INTO THE BODY OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA* (L.)¹

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Arsenical compounds have been used as insecticides for many years, yet little attention has been given to their effect as contact poisons.

This work was undertaken to determine the nature of the penetration of certain arsenical compounds through the integument of the adult American cockroach, *Periplaneta americana* (L.), when applied as a dry powder, and to learn to what extent such compounds became distributed in the insect body after penetration had taken place.

A comparison was made of the penetration and distribution of arsenious oxide and sodium arsenite.

Application was made by sealing the dry powdered toxicant in a wax cell on the metathoracic tergite of the roach. Entire roaches as well as parts and tissues were tested by means of a modified Gutzeit method to determine the amount of arsenic recoverable. The following are modifications of the method described in A. O. A. C. (1930). The generator tube was made as one unit and a paraffined cork stopper was substituted for the rubber stoppers. The initial charge in the Gutzeit generator was made up of three grams of 20 mesh granulated zinc, one millimeter of hydrochloric acid—stannous chloride solution, and 2.5 milliliters of distilled water. Also no potassium iodide was used. Instead of an aliquot the entire sample was used. The percentage of mercuric bromide was varied from 1.5 to nine. After digestion the sample was made up to nine grams with sulphuric acid. Results were recorded by means of paper strips sensitized with mercuric bromide and measured in milligrams of arsenious oxide.

Each time a series of determinations was made on solutions containing an unknown amount of arsenic, a series of determinations was also made with a number of dilutions from a standard arsenic solution made up from arsenious oxide, that would exceed any amount which might be found in the unknown. A standard graph was constructed by plotting on cross-sectioned paper the averages of the measurements from the standard arsenic solutions. Thus all results were read directly in milligrams of arsenious oxide.

Experiments were designed to determine the effect on penetration of increased area available for penetration and of varying the amounts, by weight, of the powdered arsenious oxide. When the area of the application cell was doubled the average amount of arsenic present in the insect body after 72 hours was 0.016 mg./g. body weight. In the case of cockroaches treated with the standard cell (3.2 mm. in diameter) the average

¹ Original thesis submitted June, 1936. Doctoral thesis number 388.

amount recovered was 0.006 mg./g. body weight. The difference between these two means is significant.

In experiments in which the amount of arsenious oxide was varied, the average amount of arsenic recovered after 72 hours was 0.006 mg./g. body weight when 0.01 gram was used in the cell and 0.007 mg./g. body weight when 0.03 gram was used.

A comparison was made between the penetration of arsenious oxide and that of sodium arsenite. The average amount of arsenic recovered from the bodies of cockroaches treated with arsenious oxide was, after 72 hours, 0.010 mg./g. body weight; after 120 hours, 0.025 mg./g. body weight; after 168 hours, 0.021 mg./g. body weight. With sodium arsenite the amounts were as follows: after 120 hours, 0.103 mg./g. body weight; after 168 hours, 0.162 mg./g. body weight. Sodium arsenite penetrates the integument faster than arsenious oxide.

These two toxicants vary markedly in their solubility in water. Arsenious oxide is moderately and very slowly soluble in water, Anderson and Story (1923). It is increasingly soluble in alkaline solutions due to the formation of soluble arsenites. Sodium arsenite is very soluble in water. These compounds seem to follow the same order of solubility in a fluid present on the integument of the cockroach. The arsenious acid powder became dampened after seven days in the cell and adhered to the integument. The sodium arsenite powder became dampened after a few hours and by the end of seven days became largely dissolved in the liquid present. Apparently penetration does not take place until the dry powder has become dissolved.

Results show definitely that arsenic will penetrate through the integument of a cockroach when applied either as arsenious oxide or as sodium arsenite.

Finally a comparison was made of the distribution of arsenic in parts and tissues of the adult when applied as arsenious oxide or sodium arsenite.

Groups of five cockroaches were used for dissection purposes. Tissues and parts from such a group were weighed in tared weighing bottles before they were transferred to Kjeldahl flasks for digestion.

The average weight of tissues and parts was as follows: integument 0.235 gram; legs 0.228 gram; wings 0.016 gram; digestive tract 0.079 gram; thoracic muscle 0.090 gram; central nervous system 0.005 gram.

When rather high concentrations are built up in the insect body arsenic may be recovered in all parts and tissues. Such concentrations were found when sodium arsenite was employed as the toxicant. When the less soluble arsenious oxide was used the distribution of the arsenic was practically limited to the digestive tract and to parts and tissues near the point of application.

Cockroaches which had been treated for 72 hours with arsenious oxide had an average of 0.002 mg. of arsenic in the voided feces. This, together with the fact that the largest quantity of arsenic was recovered from the digestive tract, shows that arsenic is eliminated to a considerable extent by means of the digestive tract.

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AN INVESTIGATION OF THE PENETRATION OF PYRIDINE, PIPERIDINE AND NICOTINE INTO THE BODIES OF INSECTS¹

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The problem of relative toxic efficiency of various compounds to animals has been the concern of many investigators. Among invertebrates, very few studies have followed the course of entrance of poisons into the entire body or into the tissues. This inquiry attempts to establish penetration of pyridine, piperidine, and nicotine into the body of an insect from saturated atmospheres of their vapors in air. Information is also presented on the speed of entrance and the quantity of the compounds which enter the body within a given time. Some facts are presented concerning the distribution of the compounds in the tissues.

MATERIALS

The insects used in the investigation were adults and nymphs of the American cockroach, *Periplaneta americana* (L.) and late instar larvae of the corn ear worm, *Heliothis obsoleta* Fab. The toxic compounds employed in the experiments were pyridine, redistilled from a reagent grade and collected between 112° and 113° C. (740 mm.); piperidine, C.P. (Eastman Kodak Co.), and nicotine, redistilled (99.32 per cent).

From a large number of reagents giving color reactions or precipitates with the three toxic compounds, three were selected which provided two independent tests for each of the three toxic compounds. They were Wagner's reagent, phosphomolybdic acid reagent and gold bromide reagent. Wagner's reagent detects pyridine at 0.004 gram per 100 cc. or less. Phosphomolybdic acid reagent was used to precipitate all three of the compounds. With piperidine it gives a crystalline precipitate discernible at 0.05 gram per 100 cc., and a turbidity with nicotine at 0.002 gram per 100 cc. Gold bromide reagent confirmed nicotine and piperidine at lower concentrations.

The sensitivities of various reagents were determined with aqueous solutions of the pure compounds acidified with tartaric acid. Known solutions of the compounds were tested by precipitation with the reagent. If precipitation resulted, the solution was diluted by one-half with distilled water and successively by half steps until a concentration was reached which gave a faint but unmistakable turbidity. This concentration was accepted as the limit of sensitivity of the particular compound, and it furnished the factor used in calculating the quantity of a toxic compound in the extract derived from the treated insects.

METHODS

The insects were exposed to the vapors in an ordinary gas bottle of 250 cc. capacity closed with a cork stopper. Inserted into the cork was a

¹ Original thesis submitted June, 1933. Doctoral thesis number 249.

hook from which a wire cage for the insects was suspended. An excess of the liquid compound was placed in the bottle, assuring practically complete saturation of the air around the insects. Exposure times varied with the compounds, to include ranges of no, slight and extreme mortalities. The temperature for exposure was 30° C. for the cockroaches and room temperature for corn ear worm larvae.

Since pyridine, piperidine and nicotine are volatile in steam, a steam distillation method was used to extract the compounds from the tissues. After removal from the exposure flask the insects were washed in a stream of distilled water and refluxed for 10 minutes in a Kjeldahl flask with 20 cc. absolute alcohol, acidified with tartaric acid. The alcohol was evaporated in a current of air and the syrupy residue was dissolved in 20 cc. of distilled water. This acid aqueous extract was made alkaline with dilute potassium hydroxide solution and steam was passed through the alkaline extract until 100 cc. of the distillate were collected. The distillate, acidified with tartaric acid, was reduced to 10 cc.

In determinations of various tissues or parts, the tissues were dissected out into tared weighing bottles. The muscular tissue was obtained chiefly from the leg and wing muscles; the digestive tract included the whole alimentary canal; the nerve tissue included the entire ventral nerve cord but not the brain. The fat body tissue was removed directly from the body cavity. The cuticula was the entire exoskeleton with as much as possible of the abdominal muscles and fatty tissue removed. In experiments with *H. obsoleta*, time and material permitted only the determination of pyridine in the blood. A short extraction method was used.

The standard volumes of distillates from the steam distillation of entire insects and insect tissues were diluted quantitatively and tested with an adequate reagent to determine the concentration of the toxic compound in the distillate. The quantitative values are of greater worth as comparative measures than as absolute measures of the compounds present in insects for the reagents were not necessarily employed at the exact limits of detection, but at "end-points" at which detection was certain and comparison could be made without great difficulty.

DISCUSSION

The concentration of pyridine in the bodies of cockroaches after 51 minutes when half the number was dead was 2.9 mg./g. Pyridine seemed to exhibit no great selectivity for any part or tissue. However, large quantities were found in the ventral nerve cord. Much less pyridine was present in the cockroach up to 60 minutes than in the blood of the corn ear worm, which had approximated a concentration of 11 mg./g. after 95 minutes.

About 50 per cent of the roaches had died after 12.5 minutes when the body contained a piperidine concentration of 1 mg./g. The greater accumulation of piperidine went to the muscle, cuticula and ventral nerve cord. This points to the importance of penetration through the cuticula, tissues nearest the cuticula containing the greatest quantity of piperidine. A gradient is established from the cuticula to the innermost tissues.

The nicotine concentration at which 50 per cent of the roaches had been killed was about 1.2 mg./g. and the exposure time was 14.3 hours.

Nicotine steadily accumulated in the cuticula even after the concentrations in the digestive tract and nerve tissues had reached equilibrium.

In 12.5 minutes the amount of pyridine in the body is comparable to that of piperidine, while a much smaller concentration of nicotine has been attained. Piperidine has killed 50 per cent of the insects, pyridine and nicotine, none. The rate of entrance of nicotine may be slower because the outside concentration is relatively very low, the amount vaporized penetrating as it becomes available.

Considering toxicity as a function of outside concentration as well as the concentration in the tissues and of the time, the relative toxicity of the three compounds in decreasing order is nicotine > piperidine > pyridine.

INVESTIGATION OF CODLING MOTH POPULATIONS AS THEY AFFECT CONTROL EXPERIMENTS¹

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The chief purpose of this investigation was to improve the Iowa orchardists' defense against the codling moth. In this connection attention was given to the technique of codling moth field experimentation which would not only facilitate this purpose, but also furnish information of value to other workers in the field.

As many as fifty or more factors may affect the population of codling moth on a tree at harvest. From this array the worker must select one or two for his experiment, attempting at the same time to keep the remaining variables as nearly constant as possible. That all variation can not be eliminated is obvious; therefore the worker must so replicate the units of his experiment that the uncontrolled variation may be estimated and his results relied upon proportional to the size of this random error.

The results of a banding experiment at the experimental orchard in Mitchellville, Iowa, indicate that more worms are caught on trees bearing bands on the scaffold branches in addition to one on the main trunk than on trees singly banded on the main trunk. On doubly banded trees more worms descended the branches from fruit remaining on the tree, being caught in the upper bands, than ascended from fallen fruit to be caught under the lower band.

Two years of bait trapping in Iowa orchards demonstrate the random distribution of populations from orchard to orchard. As previously suspected, however, brood flights were found to be generally earlier in the southern half of the state than in the north. The distribution of codling moth populations within an orchard as indicated by seasonal bait trap catches, demonstrates the heterogeneity of adult population from tree to tree.

Among the systematic variations of larval population may be mentioned location of fruit on a tree. From 24 trees in an orchard at Mitchellville, Iowa, all of which were treated alike, the average infestation in the tops was 18 per cent wormy and near the ground 14 per cent, a difference of four percentage units which was found to be statistically significant. Random selection of tree samples was therefore deemed important.

From 44 six-tree plots at Wenatchee, Washington, the worms per 100 apples were correlated with the percentage of wormy fruit, yielding a curved regression line fitted by the formula,

$$y = 1.049x^{1.1098}$$

with a standard error of estimate (worms per 100 apples from percentage wormy) of one worm per 100 apples for six tree means and 2.42 worms

¹ Original thesis submitted March, 1936. Doctoral thesis number 360.

per hundred apples for single trees. The correlation coefficient was 0.9092. Due to the low standard error of estimate and high correlation coefficient, counts of actual worms per 100 apples seemed unnecessary in most cases for the estimation of treatment effect.

The percentages of wormy fruit estimated with and without the inclusion of dropped fruit correlated very closely, the standard error of estimate (percentage wormy including drops from percentage wormy in picked fruit alone) being 3.45 per cent and the correlation coefficient, 0.987. The variety used in the tests was Ben Davis. In other localities and with other varieties these statistics would probably be different, but under the conditions of this experiment, the inclusion of the dropped fruits added little to the accuracy of the estimates.

A Latin square of four by four plots, each consisting of six trees, three of which were selected for harvest counts, was employed in the experimental orchard for testing differences in effect of spray treatments. From the analyses of variance and covariance it became obvious that no significant differences existed between the various plots of the orchard with respect to effect of location in the experimental block in this orchard. Neither were the differences between estimates of tree infestation based upon different sizes of samples larger than might normally be expected as long as estimates were made from purely random samples. A sample of 300 apples gave an adequate picture of tree infestation. The random variation between trees treated alike was very large and the analysis demonstrated beyond reasonable doubt that this was the major source of experimental error.

Crop size affects the infestation rather systematically and considerably, particularly between plot and test means. Small crops tend to be unusually wormy and those trees bearing large crops may be less heavily infested than normally expected. The worker must ascertain, then, whether a treatment applied to a certain set of plots is actually responsible for a low infestation or if the treatment has by chance been applied to plots bearing an unusually heavy crop which is the real cause of apparent good control. An analysis of covariance of the tests furnishes a fairly easy method of obtaining this information.

The four treatments involved were lead arsenate and lime, calcium arsenate used with a buffer of ferrous sulfate and lime, manganese arsenate plus fish oil as a sticker and a schedule employing three cover sprays of lead arsenate used against the first brood, followed by two cover sprays of summer oil and nicotine sulfate applied later in the season. The statistical analysis of the results from the tests used on the same trees in two orchards for the two seasons 1934 and 1935 indicate that the three arsenicals do not differ significantly in their effect on infestation; however, the schedule in which oil-nicotine is used against the second brood attack gives significantly better control of the codling moth. Due to the cost of this material, and the fact that it does not entirely solve the problem of excessive poisonous residue at harvest, its use can not be recommended without qualifications.

From the above outlined study of population variability it has been concluded that for the experimental conditions herein studied, a thoroughly efficient experimental analysis may be based on 300 apple samples selected at random from each tree and evaluated for percentage of

wormy fruit alone. The scoring of the dropped fruit and worms per 100 apples may be omitted without materially affecting differences due to treatment. The existence of a correlation between worminess and crop size, however, necessitates the inclusion of analysis of the covariance of these variables in reduction of data. Heterogeneity between trees within an orchard emphasizes the necessity of thorough replication.

β -HYDROXYFURANS AND SOME OF THEIR BIOLOGICAL PROPERTIES¹

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Although derivatives of hydroxyfurans are numerous and extremely important, there has been little investigation of the hydroxyfurans themselves. The availability of hydroxyfurans was considered, as well as a study of the chemical and biological properties of these compounds.

Since 3,4-dihydroxy-2,5-dicarbomethoxyfuran was more available than most of the described hydroxyfurans, this compound was used as the starting material for the preparation of the compounds investigated. Johnson and Johns² prepared 3,4-dihydroxy-2,5-dicarbomethoxyfuran by condensing diethyl oxalate with diethyl diglycollate in the presence of sodium ethoxide. These workers assigned the keto structure to the compound. Hinsberg³ used the same reaction to prepare the dimethyl ester. It was found that the 3,4-dihydroxy-2,5-dicarbomethoxyfuran gave an intense violet color with ferric chloride solution. On the basis of the ferric chloride color test, Hinsberg favored the enol form of the compound. In the present investigation enol derivatives were obtained, but as yet no keto derivatives of 3,4-dihydroxy-2,5-dicarbomethoxyfuran were obtained. Among the enol derivatives of this type, which were prepared, were 3,4-diacetoxy-2,5-dicarbomethoxyfuran (m.p. 138°), 3,4-diacetoxy-2,5-dicarbomethoxyfuran (b.p. 235°/35 mm.), the dicopper acetate salt of 3,4-dihydroxy-2,5-dicarbomethoxyfuran, 3,4-dibenzoxy-2,5-dicarbomethoxyfuran (m.p. 146°), the diammonium salt of 3,4-dihydroxy-2,5-dicarbomethoxyfuran (decomposition at 200°) and 3,4-dimethoxy-2,5-dicarbomethoxyfuran (m.p. 48°).

Glyoxal and dimethyl diglycollate were condensed in the presence of sodium methoxide. The product of this condensation was acidic in behavior and on treatment with diazomethane, a small amount of substance melting at 104°-105° was obtained. This material, when mixed with dimethyl dehydromucate (m.p. 108°-109°) in 50-50 portions, melted at 106°-108°. This reaction gave support to the postulated furan structure for the condensation product of diethyl oxalate and dimethyl diglycollate in the presence of sodium methoxide.

The effect of the alkoxide was observed when diethyl oxalate and dibutyl diglycollate were condensed in the presence of sodium ethoxide. The condensation product was 3,4-dihydroxy-2,5-dicarbomethoxyfuran (m.p. 186°).

Methylation of 3,4-dihydroxy-2,5-dicarbomethoxyfuran, using dimethyl sulfate in alkaline solution, resulted in two products. The main product was 3,4-dimethoxy-2,5-dicarbomethoxyfuran (m.p. 89.5°-90°),

¹ Original thesis submitted June, 1936. Original thesis number 385.

² Johnson and Johns, *Am. Chem. J.*, **36**, 290 (1906).

³ Hinsberg, *Ber.*, **45**, 2413 (1913).

while about 10 per cent of total yield was 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran (m.p. 150°-151°). The separation of these two substances was accomplished by extracting the 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran with a saturated solution of secondary sodium phosphate. Several enol derivatives were prepared from the 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran, as well as from the other hydroxyfurans. Among them were 3-benzoxy-4-methoxy-2,5-dicarbomethoxyfuran (m.p. 117°-118°) and 3-acetoxy-4-methoxy-2,5-dimethoxyfuran (m.p. 108°).

Hydrolysis of 3,4-dimethoxy-2,5-dicarbomethoxyfuran in alkaline solution yielded 3,4-dimethoxy-2,5-dicarboxyfuran (m.p. 243°-245°; decompn.). No hydrolysis of 3,4-dihydroxy-2,5-dicarbomethoxyfuran in alkaline solution took place. A mono acid was obtained on alkaline hydrolysis of 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran. The methoxyl group seemed to influence the hydrolysis of the ester group when on the same side of the nucleus. The product of hydrolysis from 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran was postulated as 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran (m.p. 245°-246°). This compound gives a deep violet color with ferric chloride solution. The acid chloride of 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran melted at 132°-133.5°. A negative chloralide reaction resulted with 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran.

Decarboxylation of 3,4-dimethoxy-2,5-dicarboxyfuran gave 3,4-dimethoxyfuran (b.p. 172°-174° or 94°-96°/18 mm.; D_{40}^{25} 1.1316; N_D^{25} 1.4650). 3-Hydroxy-4-methoxy-2-carbomethoxyfuran (m.p. 100°-101°), obtained by a decarboxylation of 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran, was methylated to give 3,4-dimethoxy-2-carboxyfuran (m.p. 54°-55°), which on alkaline hydrolysis gave 3,4-dimethoxy-2-carboxyfuran (m.p. 170°-171°). When 3,4-dimethoxy-2-carboxyfuran was decarboxylated, 3,4-dimethoxyfuran (b.p. 172°) was obtained. These two preparations of 3,4-dimethoxyfuran gave identical maleic anhydride addition compounds (m.p. 92°-94°) as determined by a mixed melting point. The formation of a maleic anhydride derivative indicated the presence of a diene system which would be found if the furan nucleus was present in these compounds. 3,4-Dimethoxy-2-carbomethoxyfuran also forms an addition compound with maleic anhydride which melted at 109°-111°.

Treatment of 3,4-dimethoxyfuran with stannic chloride and acetic anhydride yielded two substances—a solid (m.p. 58°-59°) and a liquid. The liquid (b.p. 160°/8mm.; N_D^{25} 1.5200) was oxidized to 3,4-dimethoxy-

2,5-dicarboxyfuran. 3,4-Dimethoxy-2,5-diacetylfuran, prepared from the diacid chloride of 3,4-dimethoxy-2,5-dicarboxyfuran and dimethylcadmium, distilled at 160°/8mm. and had a refractive index of 1.5187 at 25°.

Oxidation of 3,4-dihydroxy-2,5-dicarbomethoxyfuran or 3,4-dimethoxy-2,5-dicarboxyfuran with dilute nitric acid yielded oxalic acid. An attempt was made to produce peroxides by passing oxygen through an ether solution of either 3-hydroxy-4-methoxy-2-carbomethoxyfuran or 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran, and in each case the result was negative. These results were not in accordance with those shown by 2,4,5-triphenyl-3-furanol⁴, which formed the peroxide with ease.

⁴Kohler, Westheimer and Tishler, *J. Am. Chem. Soc.*, 58, 264 (1936).

Mercuration of 3,4-dimethoxyfuran demonstrated the extremely reactive nature of this type of compound. The only product isolated was 3,4-dimethoxy-2,5-dichloromercurifuran (m.p. 208°; decompn.). 3,4-Dimethoxy-2-carbomethoxyfuran yielded 3,4-dimethoxy-2-carbomethoxy-5-chloromercurifuran (m.p. 112°-113.5°) when treated with mercurating solution⁵.

Bromination of 3-acetoxy-4-methoxy-2-carbomethoxyfuran yielded 3-hydroxy-4-methoxy-2-carbomethoxy-5-bromofuran (m.p. 125°-127°). This compound did not give a ferric chloride color test. When 3,4-dimethoxy-2-carbomethoxyfuran was treated with bromine in carbon tetrachloride, a compound which melted at 115°-116° was obtained. The compound was postulated as 3,4-dimethoxy-2-carbomethoxy-5-bromofuran.

Reduction of 3,4-dimethoxyfuran with Raney nickel at 2750 pounds of hydrogen at 200°-205° gave a liquid which distilled at 182°-183.5°. The refractive index of this liquid at 25° was 1.4378 and the specific gravity at 25° was 1.051. Erythrane⁶ was methylated with dimethyl sulfate in alkaline solution and the product obtained distilled at 181°-183°. This liquid had a refractive index of 1.4395 at 25° and a specific gravity of 1.089 at 25°. The results from these two reactions were not satisfactory enough to claim complete identity of these two compounds.

In metabolism studies using *Aerobacter aerogenes* and zymin in a Warburg-Barcroft respirometer system 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran showed the only significant action. This action of stimulation was comparable to that of the phenols. This compound showed no action as a growth stimulant, co-stimulant or hormone when tested in higher plants.⁷

⁵ Gilman and Wright, *J. Am. Chem. Soc.*, 55, 3302 (1933).

⁶ Henninger, *Ann. chim. phys.*, (5), 7, 224 (1886).

⁷ Dr. F. W. Went of California Institute of Technology carried out these tests.

A STUDY OF THE GRAPHITIZATION OF IRON CARBIDE¹

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In the heat treatment of steel the formation and decomposition of iron carbide plays a very important role. Probably for this reason investigations were first carried out to determine its stability.

Knowing the value for the change in free energy, ΔF , of the reaction,



one can determine immediately whether iron carbide is stable or meta-stable. If the free energy of the compound is greater than the sum of the free energies of its elements, that is, if ΔF is greater than zero, the compound is meta-stable. If ΔF is less than zero, the compound is stable.

The following reactions were assumed to represent the mechanism for the graphitization and carburization of pure iron carbon compounds,

1. $\text{Fe} + 2\text{CO} \rightarrow \text{Fe}_3\text{C} + \text{CO}_2$
2. $\text{C} + \text{CO}_2 \rightarrow 2\text{CO}$
3. $3\text{Fe} + \text{C} \rightarrow \text{Fe}_3\text{C}.$

It is seen that reaction (3) is the sum of reactions (1) and (2). If ΔF_1 , ΔF_2 and ΔF_3 represent the change in the free energy of reactions (1), (2) and (3), respectively, then,

$$\Delta F_3 = \Delta F_1 + \Delta F_2.$$

Both ΔF_1 and ΔF_2 can be determined experimentally, however, the equilibrium data for equation 2 in the International Critical Tables (1) were used in this investigation to obtain ΔF_2 .

ΔF_1 was obtained by determining the equilibrium constant, K , for equation (1), and substituting in the equation,

$$\Delta F^\circ_1 = -RT \ln K.$$

Values for K were obtained at intervals of 25° from 550° to 900° C. , by reading the concentrations of CO from a curve obtained by plotting percentage CO against temperature. The concentration of CO_2 was obtained by difference. The concentrations were changed to partial pressures and these values substituted into the equation,

$$K = \frac{P_{\text{CO}_2}}{P^2_{\text{CO}}}.$$

In order to determine the equilibrium pressures of CO and CO_2 in the presence of Fe and Fe_3C , an apparatus was developed so that the

¹ Original thesis submitted June, 1936. Doctoral thesis number 376.

gases were circulated continuously by means of an electromagnetic pump through the reaction chamber and the gas analysis apparatus. The reaction chamber was a furnace made of Armco iron. In the first part of the investigation Armco iron turnings were used; in the latter part, turnings from an Armco iron carbon alloy sample containing 1.55 per cent carbon were used. The gas analysis apparatus was a conductivity meter similar to those described by Daynes (2).

The change in free energy, ΔF°_3 , for the reaction,



was obtained by adding ΔF°_1 and ΔF°_2 .

The heat of formation of iron carbide was then calculated by substituting the values of ΔF_3 into the equation,

$$\Delta H = \frac{d \frac{\Delta F}{T}}{d \frac{1}{T}}$$

and solving by a graphical method, i. e., by plotting the values of $\frac{\Delta F}{T}$

as ordinates and $\frac{1}{T}$ as abscissae. The slope of the curve so obtained gives

ΔH directly.

The calculated values for the free energy change and the heat of reaction are recorded in Table 1.

The values for the heat of formation of iron carbide as obtained by various investigators, range from $-27,500$ to $+78,750$ calories. The values calculated from the experimental data secured in this investigation indicate a range of $5,450$ to $40,700$ calories over a temperature range of 550° to 850° C.

The values obtained for the free energy change of the reaction,



vary from $+5,943$ at 550° to $-4,948$ at 900° C. Values obtained by other investigators range from $11,598$ calories to $2,281$ calories, the temperature range being from 25° to 700° C.

The results obtained by using the free energy data in the International Critical Tables indicate that Fe_3C is stable above 780° C. and metastable below this temperature.

TABLE 1. *Heat of formation of Fe₃C*

Temp. °C.	ΔF_1	ΔF_2	ΔF_3	ΔF_4
550	— 298.3	+6,241	+5,493	+34,200
575	— 188.7	+5,401	+5,212	+35,300
600	— 149.5	+4,318	+4,168	+38,000
625	— 133.1	+3,237	+3,104	+40,700
650	— 51.2	+2,105	+2,054	+34,600
675	+ 274.5	+1,076	+1,351	+35,900
700	+ 846.1	— 2	+ 844	+14,700
725	+1583.9	—1,076	+ 508	+ 5,450
750	+3017.5	—2,150	+ 867	
775	+3573.0	—3,223	+ 350	
800	+3811.1	—4,471	— 661	+29,400
850	+4030.6	—6,543	—2,512	+32,600
900	+4427.7	—9,376	—4,948	

By use of data taken from Garran (3), the results indicate Fe₃ C to be stable above 775° C. and meta-stable at temperatures below this value.

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THE VALUE OF SEVERAL ORGANIC COMPOUNDS AS CONTACT AND STOMACH POISONS FOR CERTAIN INSECTS¹

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PART ONE

THE OVICIDAL AND SCALICIDAL PROPERTIES OF SOLUTIONS OF DINITRO-O-CYCLOHEXYLPHENOL IN PETROLEUM OIL

The primary object of this part of the present investigation was to determine whether dinitro-o-cyclohexylphenol², which has shown rather unusual promise as a contact and stomach poison for certain insects, could be incorporated with a petroleum oil to obtain a more effective insecticide than the oil alone. Such a mixture of petroleum oil and dinitro-o-cyclohexylphenol would considerably reduce the amount of oil in the diluted spray, and in this way lessen the chances of tree injury without reducing the effectiveness of the mixture.

The toxicities of ammonium caseinate emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil were determined in the laboratory for eggs of a plant bug, *Lygaeus kalmii* Stål, and for San José scale, *Aspidiotus perniciosus* Comstock.

A method was given for comparing the toxicities of substances to the *Lygaeus* eggs. In the method proposed, the embryonic and post-embryonic mortalities were pooled to furnish a measure of the total effectiveness of the ovicide.

A method was given for comparing the toxicities of contact insecticides to the San José scale and other scale insects. The general design can be employed for comparing the toxicity of substances particularly in cases where the viability of the insect population is significantly variable. The statistical methods employed are very useful for testing the adequacy of the experimental technique, the homogeneity of the populations, and for ascertaining whether the departures of the results from expectancy are of a magnitude ascribable to chance. The analysis demonstrated that conclusive results could be obtained with a heterogeneous insect population provided that homogeneous groups, each of sufficient size to provide for the desired treatments, could be drawn from the population.

Lethal concentrations for emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil were established with respect to the amount of the dinitro-compound dissolved in the oil phase of the emulsions and the concentrations of oil plus the compound in the diluted sprays. The toxicities of the mixtures were represented by curves.

The results of the toxicity experiments demonstrated that only a relatively small concentration of oil was necessary to carry an effective

¹ Original thesis submitted December, 1935. Doctoral thesis number 348.

² U. S. Patent No. 1,880,404.

concentration of the dinitro-compound. For example, a 100 per cent net mortality of San José scale was obtained with an oil mixture diluted to a spray strength of 1.0 per cent and containing 3.0 per cent of the dinitro-compound dissolved in the oil phase of the emulsion. Laboratory experiments have shown that a dilution of 3.0 per cent of petroleum oil without the dinitro-compound is necessary to furnish about an equally effective mortality of the scale. In the ovicidal experiments, a 100 per cent net mortality was obtained with a 1.0 per cent dilution of the oil mixture containing 6.67 per cent of the compound dissolved in the oil phase. A dilution of 3.0 per cent oil without the dinitro-compound gave only 59 per cent net mortality of eggs. These mixtures of petroleum oil plus dinitro-o-cyclohexylphenol considerably reduce the amount of oil in the diluted sprays without reducing the effectiveness of the mixture for control of the eggs and scale.

In view of the promising laboratory and field results, the use of the mixtures for control of insects during the dormant period can be recommended.

PART TWO

THE TOXICITY OF SOME NITRO-PHENOLS AS STOMACH POISONS FOR SEVERAL SPECIES OF INSECTS

Only a few compounds whose toxicities to certain insects have been evaluated on an individual dosage basis, have compared favorably in toxicity with the arsenicals. This part of the investigation includes the results obtained with a group of nitro-phenols of which 2-4 dinitro-6-cyclohexylphenol and some of its salts have shown considerable promise as stomach poisons for insects.

The following organic compounds were administered to the insects by a leaf-sandwich method: 2-4 dinitro-6-cyclohexylphenol and its calcium, magnesium, copper, and lead salts; calcium 2-6 dinitro-4-cyclohexylphenate; calcium 2-4 dinitro-6-phenylphenate; lead 3-5 dinitro-o-cresylate. Median lethal dosages (M.L.Ds.) were estimated for those compounds that were sufficiently toxic.

2-4 Dinitro-6-cyclohexylphenol and the calcium, magnesium, copper and lead salts were found to be several times more toxic than acid lead arsenate (PbHAsO_4) to the corn ear worm, *Heliothis obsoleta* Fab. Calcium 2-6 dinitro-4-cyclohexylphenate, calcium 2-4 dinitro-6-phenylphenate, and lead 3-5 dinitro-o-cresylate were not sufficiently toxic to estimate M.L.Ds. The indications were that deviations from the structure of the lethal phenol resulted in partial or complete loss of the high toxicity to insects.

Calcium 2-4 dinitro-6-cyclohexylphenate, which was the most toxic salt examined, was about 4.4 times more toxic than acid lead arsenate to the corn ear worm, 17 times more toxic than acid lead arsenate to the armyworm, *Cirphis unipuncta* Haw., and significantly more toxic than acid lead arsenate to the cabbage worm, *Ascia rapae* L.

The speed of toxic action for 2-4 dinitro-6-cyclohexylphenol and the four salts was several times greater than for acid lead arsenate. The mean survival times for the phenol and its salts ranged from 2 to 5 hours.

Arsenic trioxide (As_2O_3), 2-4 dinitro-6-cyclohexylphenol and the calcium salt were fed quantitatively in baits to the red-legged grasshopper, *Melanoplus femur-rubrum* DeGeer. The calcium salt displayed rather low toxicity, but the phenol was 2.5 times more toxic than arsenic trioxide. Furthermore, the speed of toxic action was approximately twice that of the arsenical.

The consistent and promising results obtained with 2-4 dinitro-6-cyclohexylphenol and the four salts, appear to recommend them for practical consideration as stomach poisons for mandibulate insects.

THE PHYSIOLOGICAL ACTION OF SOME FURAN COMPOUNDS¹

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A discussion of the physiological action of several furan compounds was made. Furan itself resembles benzene in its activity. Furfural and furfuryl alcohol are quite toxic to the animal organism. 2-Furoic acid is apparently without specific action. Other compounds mentioned were: the β -diethylaminoethyl esters of several furan acids which are mild local anesthetics; a series of mixed ketones containing the furyl radical, some of which are weak hypnotics; and a group of unrelated furan derivatives which have a very disagreeable action on man. This latter group of compounds may be classified as vesicants, lachrymators, and sternutators.

In order to clear up cases of questionable orientation in the furan nucleus a need arose for furantetracarboxylic acid. It seemed likely that the synthesis of the desired acid could be effected by the enolization and subsequent ring closure of dioxalsuccinic ester. Accordingly, the ester was prepared from sodio-oxalacetic ester, which on treatment with sulfuric acid gave tetracarboethoxyfuran which could be hydrolyzed with mineral acids to yield furantetracarboxylic acid. Through decarboxylation of this acid 3,4-furandicarboxylic acid and 3-furoic acid could be secured. It appears that this method of preparation of 3-furoic acid is a more convenient source than those previously used.

At present there is no unequivocal proof for the position of the nitro group in nitrofuran. 3,4-Furandicarboxylic acid is admirably suited to give conclusive proof as to whether the nitro group enters in the α - or β -position. With both β -positions blocked an entering group would have to assume an α -position. The resulting compound could then be decarboxylated and the residuum compared with the known mononitrofuran.

It appeared that 3,4-furandicarboxylic acid in the form of its ester should undergo nuclear substitution with extreme ease because there are two α -positions available and α -positions are very reactive. But a number of attempted nitrations were carried out in which the experimental conditions were made increasingly more drastic. Either no reaction occurred or the furan nucleus was ruptured by oxidation resulting in the formation of oxalic acid. In the case of halogenation, where more strenuous conditions may be employed without accompanying breakdown of the reactants, dimethyl 3,4-furandicarboxylate was found to undergo bromination in a sealed tube at 160° in the absence of a solvent to give a compound of undetermined structure.

It appeared that the oxidation of suitable dibenzofuran derivatives would furnish furantetracarboxylic acid. An investigation of the chemistry of dibenzofuran revealed that the matter of orientation in this heterocycle was in a vastly confused state, even for the most common simple

¹ Original thesis submitted December, 1935. Doctoral thesis number 354.

substitution products. The constitution of dibenzofuran is peculiar in that the nucleus possesses two distinct conflicting directive influences for nuclear substitution reactions. The position assumed by substituents in monosubstitution of dibenzofuran is apparently governed not only by the inherent characteristics of the molecule due to the diphenyl bond and the diphenyl ether linkage, and to experimental conditions, but also by the type of entering group. Since the diphenyl bond favors the 1- and 3-positions and the diphenyl ether linkage favors the 2- and 4-positions due to their strong *o*-, *p*-directing influence, it is not surprising that the substitution reactions of dibenzofuran are highly competitive and equally as uncertain.

The orientation of nuclear substitution reactions of dibenzofuran has been reviewed. Disubstitution reactions with unlike groups have not been investigated so extensively. The constitution of the nitration product of 3-acetaminodibenzofuran was confirmed and the product was 2-nitro-3-acetaminodibenzofuran. The compound secured after hydrolysis and replacement of the amino through diazotization was found to be 2-nitrodibenzofuran. And since the nitroacetamino compound was *ortho* substituted as was shown by the formation of quinoxaline derivatives from the reduced derivatives, there could be no doubt as to 2,3-disubstitution. In a similar manner the bromination product of 3-acetaminodibenzofuran was established as 2-bromo-3-acetaminodibenzofuran. The bromoacetamino compound was hydrolyzed and aminated in a sealed tube to give an *o*-diamine as shown by its ability to form quinoxaline derivatives. The bromoamine, secured by hydrolysis, on diazotization and replacement of the diazonium group by hydrogen yielded 2-bromodibenzofuran.

A series of 3-*N*-alkylated aminodibenzofurans was prepared for pharmacological testing. The following compounds were made: dimethylamino-, diethylamino-, methylamino-, ethylamino-, propylamino-, and *N*-piperidinodibenzofuran. The Skraup reaction on 2- and 3-aminodibenzofurans produced two isomeric pyrido-derivatives in each instance. These nitrogen heterocycles as well as some of their reduced derivatives were tested for physiological action.

Other amino derivatives whose physical properties have been described elsewhere were synthesized and tested. 2-*ω*-Diethylaminoacetyldibenzofuran and 2-*ω*-piperidinoacetyldibenzofuran were secured on treating 2-chloroacetyldibenzofuran with diethylamine and piperidine. From these tertiary amines on reduction with Adams catalyst there was obtained diethylaminomethyl-2-dibenzofurylcarbinol and piperidinomethyl-2-dibenzofurylcarbinol. A reaction was effected between 2-dibenzofurylmagnesium bromide and α , β -dichlorethyl ether to give the ethel ether of chloromethyl-2-dibenzofurylcarbinol. Upon treatment of this ethoxy compound with piperidine there resulted the ethyl ether of piperidinomethyl-2-dibenzofurylcarbinol. Chloromethyl-2-dibenzofurylmethylcarbinol, prepared from the Grignard reagent of 2-bromodibenzofuran and epichlorohydrin, furnished diethylaminomethyl-2-dibenzofurylmethylcarbinol on reacting with diethylamine.

Several compounds were prepared which have not previously been reported. A Gabriel phthalimide synthesis on 4- β -bromoethyl- and 2- β -bromoethyl-dibenzofuran yielded 4- β -aminoethyl-dibenzofuran, distilling at 165°-166°/2mm., and 2- β -aminoethyl-dibenzofuran, b.p. 167°-170°/2mm.

The hydrochlorides of these amines melted at 263° and 278°, respectively. From the 4-isomer there was obtained, on treatment with methylal and acid, tetrahydropyrido-[5,4-c]-dibenzofuran, which distilled at 183°-184°/1-2mm. and whose hydrochloride melted at 259°.

2-Chloromethyldibenzofuran was prepared by saturating a petroleum ether (b.p. 75°-115°) solution of dibenzofuran with hydrogen chloride in the presence of zinc chloride and trioxymethylene, and melted at 78.5°-79.5°. This halogen compound was converted to 2-cyanomethyldibenzofuran in the customary manner, and was found to melt at 102.5°-103.5°. Reduction of the nitrile with Adams catalyst yielded 2- β -aminoethyldibenzofuran.

The pharmacological reports revealed that the dibenzofuran derivatives investigated in this study are relatively toxic and that they possess physiological action of meager therapeutic importance. It is, however, significant that the 4-aminodibenzofuran and 4-acetaminodibenzofuran show analgesic action.

A STUDY OF SOME LIPOLYTIC MICROORGANISMS ISOLATED FROM DAIRY PRODUCTS¹

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The ability of an organism to hydrolyze fat appears to be an important character and in the future will be considered along with the other characters in preparing descriptions of species. The need for simple and reliable methods of determining lipolysis is therefore apparent. The objects of the investigation were to consider some of the methods used for the detection of bacterial lipolysis and to isolate and study a number of lipolytic microorganisms commonly found in dairy products.

SECTION I. OBSERVATIONS ON THE METHODS USED FOR THE DETECTION OF BACTERIAL LIPOLYSIS

The Nile blue sulfate technic consisted of the addition of 0.5 ml. of a fat emulsion (2 per cent cottonseed oil in 0.5 per cent agar) to each plate before pouring with beef infusion agar containing Nile blue sulfate in the proportion of 1 part dye to 10,000 parts of the agar; lipolysis was detected by a change in color of the globules in the vicinity of lipolytic colonies from pink to blue. Due to the inhibition of many non-lipolytic organisms, this technic was found useful for the isolation of lipolytic types when they were present in small numbers as compared with the total numbers. However, a disadvantage of the method was that certain lipolytic types were also inhibited.

The modified Nile blue sulfate technic was carried out by growing the organisms on plates containing dispersed cottonseed oil or butter fat and, after incubation, flooding the plates for 30 minutes with a 1 to 1,500 aqueous solution of the dye. Globules in the vicinity of lipolytic colonies were stained blue while those at a distance were stained pink. The modified method was very valuable when total and lipolytic counts were desired. However, when the proportion of lipolytic organisms to total organisms was low the modified method was not especially useful.

The simple triglyceride technic consisted of dispersing tripropionin or tributyrin in agar used for growing organisms and detecting lipolysis by a disappearance of the globules. The simple triglyceride technic was not an accurate method for determining lipolysis since some cultures of *Streptococcus lactis* were found to hydrolyze tripropionin and tributyrin but not cottonseed oil or butter fat.

In the natural fat technic emulsified cottonseed oil was added to agar used for growing the organisms and lipolysis was detected by a change in the appearance of the fat globules. The technic was well adapted to the study of lipolysis because relatively high total and lipolytic counts could be obtained and the picking of lipolytic colonies was not complicated by

¹ Original thesis submitted June, 1936. Doctoral thesis number 381.

flooding the plates. A disadvantage of the technic was the impossibility of detecting lipolytic bacteria when they were present in small numbers as compared to the total numbers.

SECTION II. DISTRIBUTION OF LIPOLYTIC MICROORGANISMS IN DAIRY PRODUCTS

Lipolytic bacteria were found to be distributed widely in both normal and abnormal samples of milk, cream and butter. Many of the samples of these products, especially if they were held at comparatively low temperatures for extended periods, yielded cultures of *Pseudomonas fragi*.

Attempts to isolate lipolytic microorganisms from milk obtained from the vat immediately after pasteurization invariably resulted in failure although they had been shown to be present before heating. This suggests that the presence of lipolytic organisms in pasteurized dairy products is due to contamination after heating.

In addition to the materials already mentioned the plating of miscellaneous dairy products and various other materials yielded numerous cultures of lipolytic microorganisms.

SECTION III. THE IDENTIFICATION AND CLASSIFICATION OF CERTAIN OF THE ORGANISMS ISOLATED

A large number of lipolytic cultures were isolated during the plating of various dairy products and miscellaneous materials.

Most of the lipolytic cultures obtained were apparently *Ps. fragi*. Fifty-eight of the cultures thought to be *Ps. fragi* were studied in detail and found to agree with the organism described by Hussong (3). *Ps. fragi* was found to be widely disseminated and was one of the organisms encountered most frequently in the spoilage of certain dairy products. The defect produced was either a typical rancidity or an odor suggesting *Ps. fragi*.

A new lipolytic species, *Achromobacter oleifindens*, was isolated and described. *Ach. oleifindens* differed from the more common lipolytic species because of the acid coagulation of litmus milk and of the failure to digest milk. The organism was not proteolytic and did not produce rancidity in butter.

A number of inert lipolytic cultures were obtained and were considered identical with *Bacillus abortus* var. *lipolyticus* described by Evans (1). A study of the morphology, cultural characters, and biochemical features of the organism permitted an extension of the description given by Evans. The characters of the species indicated it belonged in the genus *Alcaligenes* and the name *Alcaligenes lipolyticus* was proposed. The organism produced rancidity in butter and was characterized by its ability to rapidly hydrolyze fat and to use salts of certain of the fatty acids as the sole source of carbon.

A number of cultures of a yeast that was lipolytic, as well as proteolytic, were studied and found to be *Mycotorula lipolytica*, which was investigated by Harrison (2). The cells of this organism were ellipsoidal to cylindrical with occasional hyphal-like threads. The yeast attacked fat readily and some cultures produced rancidity in butter while others produced cheesiness. The organism grew readily on ordinary laboratory media.

The results obtained on the cultures studied indicated that even such a character as the ability to hydrolyze fat may not be stable. This was especially true with certain of the cultures of *Ps. fragi*. The failure of some of the lipolytic cultures studied to produce rancidity in butter was thought to be due to poor growth or no growth in butter; in some cases when an organism was proteolytic as well as lipolytic a cheesy condition rather than rancidity resulted.

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FEASIBILITY OF CERAMIC PRODUCTS AS TRICKLING FILTER MEDIA¹

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Trickling filters are beds of rock or other materials on to which sewage or other waste is sprinkled or otherwise distributed and through which it trickles or percolates in the process of purification. Purification is brought about largely as a result of the biological life which is present in the jelly-like slime or film which soon develops on each individual piece of filter medium.

It has been held that the operation of the filter is dependent upon the effective surface of this microbial film, and the ease with which the oxygen of the air can reach all parts of this film. If this is true then specially preformed material having especially large interstitial spaces and a maximum amount of surface per unit of volume will have marked advantages. Ceramic products such as absorption tower packing offer a maximum surface as well as a maximum interstitial space. With this in mind the feasibility of using ceramic products as trickling filter media was investigated.

In a preliminary experiment two filters, each four square feet in area and six feet in depth, were dosed intermittently with a synthetic waste. One to three-inch granite and one-inch Raschig rings were used as the media in the two filters. The rate of application was maintained constant at 2 M.G.A.D. with a six minute dosing cycle. A synthetic waste was produced continuously from dried sheep manure and later from a mixture of dried sheep manure and spray dried skim milk powder. On the basis of concentration of applied waste the operation of this experimental plant may be divided into three periods. During the first period from May 23 to June 20, 1933, the average B.O.D. concentration of the applied waste was 117 p.p.m. and the effluents from the Raschig ring and granite filters contained 18 and 21 p.p.m., respectively. During the second period from June 20 until August 25, the average concentration of the applied waste was 567 p.p.m. and the effluents were 46 and 58 p.p.m. for the Raschig rings and granite, respectively. During the third operating period from August 25 until September 28, the average concentration of the applied waste was 999 p.p.m., although at times the concentration was as high as 1400 p.p.m. The concentrations of the effluents obtained were 146 and 309 p.p.m. for the Raschig rings and granite, respectively.

In spite of the high concentration and rate of application of the waste the filters at no time clogged to the extent of making remedial measures or stoppage of the filters necessary, although the granite did, at times, pond.

In this preliminary experiment the Raschig ring filter showed better B.O.D. reduction, more uniform operation and greater nitrification than

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the granite control filter. Dried sheep manure was not found satisfactory for the production of a uniform concentration of "synthetic" waste.

Seven different filter media were placed into filters, each of four square feet area and six foot depth. The filter media used were one to three-inch granite, $\frac{3}{4}$, 1, $1\frac{1}{2}$, and $2\frac{1}{4}$ -inch Raschig rings, a special block designated as Straight's block and corn cobs. The ceramic media used in this and in the preliminary investigation were produced from Iowa clays. These experimental filters were placed into operation at the sewage disposal plant of the City of Ames, and were dosed with settled sewage from the effluent from the settling chamber of the city Imhoff tank. The filters were dosed at a very constant rate by means of a motor operated dosing device. Chemical analyses were made on 24-hour and bi-weekly chloroformed composite samples of both the influent and the effluents.

The operation of this experimental plant may be divided into three operating periods on the basis of the rate of application of the sewage. During the first operating period from August 16 until December 9, 1934, the rate of application was 2 M.G.A.D. with a six minute dosing cycle. During the second operating period from December 9, 1934, until April 1, 1935, the rate of application was 4 M.G.A. D. with a 3 minute dosing cycle. During the third operating period from April 1 until June 18, the rate of application was 8 M.G.A.D. with a 3 minute dosing cycle. The experimental plant has been continued in operation since the third operating period at a rate of 16 M.G.A.D. with a 3 minute dosing cycle.

The average B.O.D. concentration of the influents during each of the three operating periods was about 190 p.p.m. At no time during the operation of the plant did clogging or ponding occur. During the first two operating periods the effluent from the three-fourths-inch ring contained the least B.O.D. with the larger sizes of rings containing progressively greater amounts. During these two periods of operation the B.O.D. of the effluent appeared to be a function of the surface of the filter media. During the third operating period the effluents from all the filters contained nearly the same concentration of B.O.D., with a slight advantage in favor of the larger rings. It was postulated that during this period the ventilation obtained in the filters became the limiting factor in B.O.D. removal and that as a result the larger ring filters, since they offered less resistance to natural ventilation, were able to produce an effluent of lower B.O.D. concentration.

An unusual amount of nitrification was obtained in the three-fourths-inch ring filter. Average nitrate concentration of 18.6, 17.0, and 14.0 p.p.m. as nitrogen were obtained during the three operating periods from this filter. Progressively smaller amounts of nitrates were obtained from the larger sizes of rings. It was found that throughout the operation of the filters the nitrification obtained appeared to be a function of the surface of the media.

There was some evidence presented to show that the limiting factors for nitrification and B.O.D. removal are not the same.

Studies were made of the rates of runoff from the filters for various rates of application and various dosing cycles, both before and after the microbial film had developed. It was found that the microbial film had a very marked effect in smoothing out the flow through the filter. It was found that the flow of water through the filters was markedly different

than that of sewage. It was shown that this difference was in part caused by the difference in surface tension of water and sewage.

The least variation in runoff rates occurred in the filters having the smallest medium. That is, the larger surface of the medium serves to delay or impede the flow through the filter and to smooth out the flow.

After the microbial film had developed the variation in rate of runoff from the three-fourths-inch ring filter was found to be very small. Inspection of the curves obtained after the film had developed indicates that dosing cycles of less than 3 minutes are desirable, especially at the higher rates of waste application.

An estimate is presented for the cost of manufacture of Raschig rings. It appears probable that one-inch Raschig rings can be produced under competitive conditions for less than \$5.00 per cubic yard. Further, it appears probable that Raschig rings can, because of the greater capacity and greater efficiency obtained, successfully compete with the materials now commonly used for trickling filter media.

THE RELATIVE REACTIVITIES OF SOME ORGANOMETALLIC COMPOUNDS¹

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In organic synthesis the use of selective or preferential reactions with compounds having polyfunctional groups is of very great importance. The determination of the relative rates of reaction of members of groups or classes of compounds with certain functional groups affords a method for estimating the proper reagents to use in reactions where more than one functional group is involved. In this study an attempt has been made to compare the relative reactivities of the organometallic compounds of aluminum, boron and zinc with several selected reactants. It was hoped that by the use of these less reactive organometallic compounds new syntheses could be realized with polyfunctional compounds not otherwise attainable with the more reactive organometallic compounds.

The color test for reactive organometallic compounds was used in these rate studies. The color test, as originally developed, was used only with the more reactive organometallic compounds formed from metals in the first and second groups of the periodic table. Positive color tests were obtained by the reaction of the organometallic compounds of aluminum, boron and zinc on Michler's ketone provided fairly concentrated solutions, higher temperatures and longer periods of contact were used. In order to obtain a good color test with these less reactive organometallic compounds it was found necessary to use solutions of approximately 1 molar concentration. More dilute solutions were either heated at 100-110° for 15 minutes, as in the case of the less reactive organozinc compounds, or allowed to stand with Michler's ketone for as long as 48 to 60 hours. This suggested the possibility that other reactions which do not go under ordinary conditions may proceed very slowly and require considerable time for completion. In this connection several organometallic compounds of Hg, Pb, Sn and Bi were sealed in test tubes with a 1 per cent Michler's ketone solution to determine if after prolonged contact reaction had taken place.

A survey of the literature shows that practically the only reactions of organo-aluminum compounds reported were condensation reactions. In most cases reported concentrated solutions of the mixed organo-aluminum compounds were reacted with different functional groups. In this work more dilute solutions of the simple organo-aluminum compounds were studied. Several reactions of tri-*p*-tolylaluminum, triphenylaluminum and tri-*n*-propylaluminum have been investigated and found to proceed in the normal manner of the more reactive organometallic compounds but at a slower rate. Thus, triphenylaluminum gave 23.1 per cent yields of triphenylcarbinol after standing 16 days at room temperature with benzophenone. Similar reactions were carried out between organo-aluminum

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compounds and phenyl isocyanate, benzaldehyde, carbon dioxide and benzonitrile. The use of xylene solutions for the preparation and reactions of organo-aluminum compounds was found to be very advantageous.

The reactions of organoboron compounds were also investigated. No reactions with functional groups have hitherto been reported, and the only reactions entered into by these compounds were oxidation, hydrolysis, ammination and the addition of alkali metals. The reaction of triphenylboron with phenyl isocyanate and benzaldehyde was investigated and the expected products were isolated in 16.2 per cent and 10 per cent yields, respectively. The products were not isolated from the reaction of triphenylboron with benzophenone or benzonitrile. It was not possible to isolate the expected products from the reaction of tri-*n*-propylboron with various functional groups. Several difficulties were experienced in working with organoboron compounds and the field contains many interesting problems. The preparation and purification of these organoboron compounds has been described.

The reactions of organozinc compounds are very numerous, but, in general, these compounds are considered as not reacting with the carbonyl linkage. Diphenylzinc has been found to react with carbon dioxide, benzophenone, phenyl isocyanate and benzonitrile. The reaction of di-*n*-propylzinc with phenyl isocyanate was the only reaction tried using the alkyl compounds, and since the normal product was obtained here further studies were not made.

To determine the relative reactivities of these different organometallic compounds 50 cc. of a 0.2 molar solution of these compounds in xylene was treated with a 10 per cent excess of the reactant. Two cc. portions were removed at regular intervals and tested for the presence of organometallic compounds. The time required for the completion of the reaction was determined in this manner for each organometallic compound. In this manner the reaction rates of the phenyl derivatives of aluminum, boron and zinc were compared with one another and with the *n*-propyl and *p*-tolyl derivatives of the same metals. Benzaldehyde, benzophenone and benzonitrile were the reactants used. In general, the organo-aluminum compounds were most reactive, the organoboron compounds were next, and the organozinc compounds least reactive with the reactants studied. The *n*-propyl and *p*-tolyl derivatives appeared to be more reactive than the phenyl derivatives. The relative order of decreasing reactivities of the various functional groups studied seemed to be the same as previously reported; that is, benzaldehyde, benzophenone and benzonitrile.

The minimum concentration of the phenyl and *n*-propyl derivatives of aluminum, boron and zinc which were required to give a positive color test was determined and the relative rates of reaction of dilute solutions of these derivatives with Michler's ketone were observed. In each case the order of decreasing reactivity seemed to be: organo-aluminum compounds, organoboron compounds, organozinc compounds.

The preparation of organo-aluminum and -zinc compounds by the displacement of mercury from organomercury compounds by aluminum and zinc, respectively, affords a method of comparing these two organometallic compounds. By use of a qualitative test for organomercury compounds the length of time required for the disappearance of organomercury compounds in these solutions was determined. It was found that in a

boiling xylene solution aluminum would replace mercury in approximately $2\frac{1}{2}$ hours, while zinc required 6 to 8 hours.

Some rather definite correlations have been made between the relative reactivities of the organometallic compounds and the position of the metal in the periodic table and in the electrochemical series.

SUMMARY

1. The color test for reactive organometallic compounds has been applied to the lesser reactive organometallic compounds.
2. The preparation and reactions of organometallic compounds of aluminum, boron and zinc have been studied.
3. The relative rates of reaction of the *n*-propyl and phenyl derivatives of aluminum, boron and zinc with Michler's ketone, benzaldehyde, benzophenone, and benzonitrile have been studied.
4. The order of decreasing reactivities has been found to be: organo-aluminum compounds, organoboron compounds, organozinc compounds.
5. A correlation has been made between the relative reactivities of some organometallic compounds and the position of the metals in the periodic table and in the electrochemical series.
6. The probability of the preparation of otherwise inaccessible compounds through the medium of selective or preferential reactions involving these less reactive organometallic compounds has been discussed.

THE DECOMPOSITION OF SOME HUMUS-FORMING MATERIALS IN SOILS¹

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Experiments were conducted to study the influence of oat straw, wheat straw, sudan grass, cane sorghum, flax, cornstalks, millet, hemp, soy beans, alfalfa, sweet clover, and red clover on the numbers of cellulose decomposing organisms in the soil. It was observed that each material brought about an increase in the number of cellulose decomposing organisms but there was no consistent difference in number of organisms in the soils treated with the different plant materials.

The respiration chamber method was used for measuring carbon dioxide production in soils. A film was found to form over the surface of the barium hydroxide in the presence of carbon dioxide. Preliminary studies showed that agitating the barium hydroxide in the bottom of the chamber did not give any increase in the amount of carbon dioxide absorbed. It was concluded, therefore, that all of the carbon dioxide was absorbed when the chambers were stationary. An analysis of the atmosphere within the chamber during the progress of an experiment indicated that the oxygen content was normal and that there was no accumulation of carbon dioxide in the air of the chamber. The respiration chamber method for measuring carbon dioxide production in soils was preferred to the aspiration method as the respiration chamber method required less equipment and less time for the determinations than the aspiration method.

The evolution of carbon dioxide from the soil treated with the different plant materials was measured by the chamber method during 190 days. The results obtained showed that the materials containing the most nitrogen produced the most carbon dioxide during the first few days of the experiment, but after this time the materials containing small amounts of nitrogen produced the most carbon dioxide. The various plant materials listed in descending order of the amount of carbon dioxide produced after 228 hours are sweet clover, alfalfa, soy beans, flax, red clover, millet, hemp, sudan grass, cane sorghum, cornstalks, oat straw and wheat straw. Plotting the logarithm of the mean carbon dioxide evolved from the soils treated with non-legumes against the logarithm of time from 15 days until the end of the experiment gave a straight line. The slope of the line for the non-legumes was greater than the slope of the line for the legumes.

Soils treated with the plant materials at the rate of 0.3 per cent and 4.0 per cent showed a nitrate depression directly related to the nitrogen content of the plant materials added. An *r* value of 0.95 was obtained with the 0.3 per cent treatments and an *r* value of 0.99 was obtained with the 4.0 per cent treatments. This indicates that under favorable soil conditions the rate of organic matter decomposition is significantly correlated with the nitrogen content of the organic matter.

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The nitrogen content of these plant materials varied between wide limits, but the carbon contents varied little. The different plant materials were found to differ greatly in base exchange capacity. The base exchange capacity of the various materials was correlated with the nitrogen and lignin content, but not with the alcohol benzene fraction or the hydrolyzable fraction. The base exchange capacity of the different plant materials increased as the materials decomposed. The increase in exchange capacity was found to be significantly correlated with the increase in lignin content. Many of the plant materials did not differ greatly in exchange capacity after decomposition but, in general, as decomposition proceeded the leguminous plant materials continued to possess a higher base exchange capacity than the non-leguminous plant materials. The difference became less as decomposition proceeded. As the plant materials decomposed the exchange capacity increased much more rapidly than the lignin content or the decrease in weight. At the end of 210 days there was no definite relation between the exchange capacity and the lignin content, the alcohol-benzene-soluble fraction or the amount of hydrolyzable material.

Base exchange studies were made on soils treated with the different plant materials at the rate of 5.0 per cent, and which had been allowed to decompose for 289 days under laboratory conditions. The results showed that the base exchange capacity of the soils was significantly correlated with the nitrogen content of the plant materials added. The soils receiving plant materials of high nitrogen content were highest in the base exchange capacity.

The base exchange capacity of soils treated with 4 per cent of the different organic materials was determined at intervals as the materials decomposed. The results obtained showed an increase in exchange capacity in each case. Some of the plant materials increased the exchange capacity of the soil more than others. Three plant materials apparently reached a maximum in base exchange capacity before the last sampling as the exchange capacity was lower than at the previous sampling.

Analyses of the undecomposed and decomposed plant materials showed that the lignin content increased as decomposition proceeded. However, the lignin content of the decomposed plant materials calculated on the basis of the original organic matter showed a significant decrease in the lignin content. The losses in lignin were found to range from 24.71 per cent with cane sorghum to 54.80 per cent with soy beans.

THE ELECTRON-SHARING ABILITY OF ORGANIC RADICALS. THE TERPENES AND RELATED COMPOUNDS

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The modern electronic theory of valence seems to offer the most adequate explanation of the properties of organic compounds available at the present time. According to this theory the properties of a molecule are dependent upon its electronic configuration and these properties may be varied by shifting this electronic configuration. The introduction of a substituent into an organic molecule, therefore, causes a change in the properties of the molecule because of a difference in the ability of the substituent to share electrons with, or transfer them to or from, the atoms to which they are linked. One of the most readily determined properties of the organic acids and amines is their apparent degree of ionization.

Ostwald was the first to observe that the ionization constant of organic acids changed by a definite amount when a substituent was placed in the same position relative to the carboxyl group. Wegscheider extended the investigations of Ostwald and summarized the available data into tables of factors which represented the effect of the position of various substituents upon the dissociation constants of organic acids. This "Ostwald Law" has been the basis for a great deal of experimental work.

Derick attempted to establish a standard for determining the effect of introducing a radical into a molecule. He attempted to use the acidic and basic dissociation constants of certain hydroxides. Because of the apparent lack of ionization in so many compounds this standard was difficult to apply experimentally.

Hixon and Johns and co-workers have demonstrated a mathematical relationship which places organic radicals attached to a polar group in a definite order in a series. This relationship for the acids and amines was of the type:

$$\text{Log } K = ke^{ax+b} + C$$

where $\log K$ is the logarithm of the dissociation constant and x is the abscissa value, or "electron-sharing ability," of the radical. The radicals considered must contain no polar group themselves. It was further pointed out that the electron-sharing ability appeared to be a function of the mass of the radical and the spatial configuration of the molecule as well as the potential of the atoms.

The investigation presented here was undertaken to determine the ionization constants of some terpene amines and to predict, if possible, some types of structure the amines of which might have dissociation constants in the range 10^{-5} - 10^{-9} . Qualitative observations were made on the degree of stability of the amines studied.

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The hydrochlorides of the following amines were prepared and purified:

- (1) Bornyl amine
- (2) Pinyll amine
- (3) α -Aminoamyl methyl ketone
- (4) Camphor amine

1-Menthyl amine was obtained from Eastman Kodak Company and its hydrochloride purified.

The ionization constants of the amines just mentioned were measured by a modification of the half neutralization method. The data obtained are tabulated in the accompanying table.

Tabulated Results from E.M.F. of Cells of Half Neutralized Amines

Pd/H₂(1 atm.) amine sol./KCl(sat.), Hg₂Cl₂/Hg

Substance	Wt. of Amine. HCl	cc.'s NaOH 0.01586N	Pres- sure mm. Hg	E.M.F. Obs.	E.M.F. 760 mm.	-log K _B
1-Menthyl amine	0.0859 ¹	14.12	738	0.8478	0.8486	3.81
	0.1023 ¹	16.85	738	0.8489	0.8497	3.79
Bornyl amine	0.1025 ²	17.04	734	0.8290	0.8299	4.17
	0.0410 ¹	6.82	742	0.8294	0.8302	4.12
Pinyll amine	0.0837 ¹	14.06	734	0.7522	0.7531	5.42
	0.1002 ¹	16.84	734	0.7521	0.7530	5.42
α -Aminoamyl methyl ketone	0.0487 ³	9.27	737	0.7287	0.7296	5.82
	0.0609 ³	11.55	731	0.7300	0.7310	5.80
	0.0543 ³	10.34	726	0.7302	0.7313	5.79
Camphor amine	0.2065 ²	18.13 ⁴	742	0.6644	0.6652	6.91
	0.2052 ²	18.02 ⁴	742	0.6697	0.6705	6.82
	0.2036 ²	17.88 ⁴	742	0.6644	0.6652	6.91
	0.2046 ²	17.96 ⁴	742	0.6611	0.6619	6.96

¹ Made up to 100 cc. solution.

² Made up to 250 cc. solution

³ Made up to 50 cc. solution.

⁴ Normalcy 0.02797.

Some observations upon the structures of the amines studied in this investigation might prove of interest. Camphor amine, with an ionization constant of 1×10^{-7} , was the most negative. Bornyl amine, with a similar structure, proved to be much more positive ($K = 7 \times 10^{-5}$). Even though the amino group is on the 4-position in the first case and on the 3-position in the second, the only major difference between the two compounds is the presence of a carbonyl group in camphor amine. The larger part of the difference in the ionization constants of these two compounds must be due to the negativity of this grouping. This contention is further strengthened by the constant obtained for α -aminoamyl methyl ketone

(1.6×10^{-6}). All previous data obtained in this laboratory indicate that, if the oxygen in the above compound be replaced by two hydrogen atoms, the magnitude of the ionization constant should increase to about 10^{-4} . The ring structure of camphor probably accounts for the lower ionization constant of its amine. The effect of the bridge-ring is also observable in the cases of menthyl and bornyl amines. Pinyll amine illustrates the effect of the four membered bridge-ring as contrasted with the five membered ring of the bornyl radical. The methylene group one carbon removed from the amino radical without doubt contributed to the negativity of the pinyll structure.

Some bicyclic terpene amines containing a three membered ring may possibly prove still more negative.

CONCLUSIONS

From the data presented in this study the following conclusions may be drawn:

1. The terpene amines have ionization constants ranging from about 10^{-4} to 10^{-7} .
2. There is evidence of instability in those amines with constants of the order of 10^{-6} to 10^{-7} .
3. There is qualitative evidence indicating the existence of a minimum stability range in the electron-sharing ability curve for amines.
4. The instability of primary amines of the terpene type explains the lack of values in the literature for ionization constants ranging from 10^{-5} to 10^{-9} .
5. So-called aliphatic amines may be modified by placing a carboxyl group in the alpha position, to give ionization constants and stabilities within this unstable range.
6. Ionization constants may be determined by a modification of the half neutralization method, using the amine hydrochloride and a half-equivalent of NaOH instead of the usual procedure.

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PHYSIOLOGY OF THE LACTIC ACID BACTERIA¹

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The true lactic acid bacteria constitute a large and ubiquitous group of microorganisms forming large quantities of lactic acid from carbohydrates. The group is conveniently subdivided into homofermentative and heterofermentative bacteria. The former produce substantially nothing but lactic acid from glucose with essentially traces of carbon dioxide, acetic acid and glycerol, whereas the hetero-forms yield relatively large quantities of ethyl alcohol, carbon dioxide, acetic acid and glycerol in addition to lactic acid.

Relatively few investigations have dealt with the dissimilation of carbohydrates by the lactic bacteria, in particular in the case of the hetero-lactic forms.

A careful study of the dissimilation of carbohydrates brought about by the lactic bacteria should prove of value to agriculture, the fermentation industry and to systematic bacteriology.

The present investigation involves primarily a study of the mechanism of the dissimilation of glucose and levulose by identified and well known cultures as follows: *Lactobacillus lycopersici*, *L. mannitopoeus*, *L. plantarum*, *L. acidophilaerogenes*, *L. cucumeris*, *L. gracilis*, *L. fructivorans* and *Leuconostoc dextranicus*.

Quantitative studies of the dissimilation of glucose and levulose were made; intermediary products were determined by the addition of fixatives and the breakdown of the intermediaries was studied.

The quantitative relationships established among the final products lead to certain conclusions as to the mechanism of dissimilation.

The fermentation of glucose by both homo and hetero bacteria yielded acetic acid and carbon dioxide in equimolar quantities. It is probable that these two compounds originated from the breakdown of a single 3-carbon intermediary. The formation of acetic acid and carbon dioxide from the secondary fermentation of lactic acid by hetero lactic forms is suggested by the results. Pyruvic acid would be a precursor of the 2- and 1-carbon compounds according to the Embden-Meyerhof scheme of muscle and yeast dissimilation. This compound was fermented to acetic acid, carbon dioxide and lactic acid. However, pyruvic acid was isolated as an intermediary in the aerobic breakdown of lactic acid. The reaction between pyruvic acid and lactic acids may prove to be reversible.

The formation of carbon dioxide and acetic acid is an oxidative change and must be accompanied by a reduction product. This requirement was met by the formation of glycerol equal to twice the acetic acid.

Ethyl alcohol, formed by the hetero-group, was accompanied by equimolar quantities of carbon dioxide, indicating the formation of the alcohol by the breakdown of a 3-carbon intermediary. Evidence of this assumption

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tion was shown by serial analyses of glucose fermentations. The percentage of glucose fermented to lactic acid decreased while that fermented to acetic acid, carbon dioxide and ethyl alcohol increased. The latter three compounds are thus formed at the expense of the lactic acid either by the breakdown of lactic acid itself or by an increase in the rate of their formation from a precursor of lactic acid. The formation of ethyl alcohol by the decarboxylation of lactic acid has never been shown, to the knowledge of the author. According to the Embden-Meyerhof theory, ethyl alcohol is formed by the reduction of acetaldehyde, the latter resulting from the decarboxylation of pyruvic acid. Although ethyl alcohol was not formed from pyruvic acid fermented by lactic acid bacteria, it is probable that conditions in the fermentation of glucose were such that they brought about the formation of ethyl alcohol from intermediary pyruvic acid.

The addition of certain hydrogen acceptors to fermentation of glucose may divert the normal course of dissimilation and thereby throw some light on the dissimilative mechanism. Acetaldehyde and acetyl-methylcarbinol added to fermentations of glucose by heterofermentative bacteria resulted in a decrease in ethyl alcohol, lactic acid and glycerol and an increase in acetic acid and carbon dioxide. The added hydrogen acceptors were reduced, the acetaldehyde to ethyl alcohol and the acetyl-methylcarbinol to 2,3-buteneglycol. Added hydrogen acceptors may be expected to compete with hydrogen acceptors formed by the dissimilation of glucose. It appears that ethyl alcohol, lactic acid and glycerol are formed by the reduction of intermediary hydrogen acceptors.

Levulose plays two roles in fermentations by the heteroforms; (a) part is fermented to acetic acid, carbon dioxide, lactic acid and ethyl alcohol, and (b) part acts as a hydrogen acceptor and is reduced to mannitol. If the quantity of levulose changed to mannitol is subtracted from the total quantity of levulose fermented and the products calculated on the basis of the difference, the relationships are very similar to those obtained by fermentations of glucose to which hydrogen acceptors have been added.

The present work may be interpreted as supporting both the generally accepted scheme of dissimilation among bacteria involving methylglyoxal as a 3-carbon intermediary and an adaptation of the Embden-Meyerhof scheme of muscle glycolysis in which phosphoglyceric acid and pyruvic acid play an important part. The data obtained do not permit acceptance of one scheme to the exclusion of the other. In view of the many mechanisms that may bring about the end-products in the same quantitative relationships, and the isolation of intermediary compounds that do not satisfy a single scheme of dissimilation it is probable that no one of the present schemes governs fermentation under all conditions. Further study should conciliate the various theories of carbohydrate breakdown and clarify the roles played by intermediary compounds such as methylglyoxal and phosphoglyceric acid.

THE EFFECT OF PHOSPHATE FERTILIZERS ON SOIL REACTION¹

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Experiments were carried out to study the effect of different phosphate fertilizers on the reaction of Carrington loam, Carrington silt loam, Tama silt loam and Grundy silt loam. The soils were sieved, treated with different phosphate fertilizers, mixed well, potted and kept in the greenhouse under uniform conditions. Samples were taken periodically for laboratory analysis.

An analysis of variance of the data for 10 samples of the Carrington loam taken over a period of eight months showed that 120 pounds per acre of superphosphate decreased the pH to a highly significant extent. Two hundred forty pounds per acre caused a highly significant lowering of pH below that resulting from the 120 pound application. One thousand pounds per acre of rock phosphate increased the average pH above that of the untreated soil by an amount greater than the least mean difference which could be considered highly significant and 2,000 pounds per acre resulted in a further highly significant increase in pH. The exchangeable hydrogen was highly significantly correlated with pH, the correlation coefficient being -0.9644 . No significant difference was found in the base exchange capacity of the variously treated soils.

It is interesting to note that although the increase in mean pH from 5.79 to 5.85 as the result of applying 2,000 pounds of rock phosphate per acre was highly significant statistically, it was still very much below the pH value of 7.00 produced by 4,000 pounds of limestone, the amount needed to neutralize this soil according to the Truog test.

A second study was made of the effect of rock phosphate and sodium phosphate, each applied alone and in combination with lime, and of lime alone on the reaction of Grundy silt loam in the greenhouse. A statistical analysis of the data from 13 samplings of duplicate pots taken over a period of 20 months in which the soils were uniformly watered but not cropped showed that 500 pounds or more per acre of rock phosphate decreased the acidity by a highly significant quantity as measured by pH and by the Hardy and Lewis lime requirement method. Higher rates of rock phosphate seemed to be more effective in neutralizing acidity of the soil. However, rock phosphate did not produce a significant difference in the amount of exchangeable hydrogen in Grundy silt loam or in the base exchange capacity.

In order to get some idea of the effect on plant growth of the neutralizing value of rock phosphate a sweet clover crop was grown in a series of pots of Grundy silt loam treated the same as in the fallow experiment described above. Applications of 500 pounds per acre or more of rock phosphate resulted in highly significant increases in yields of sweet clover, the two highest rates of application, namely, 2500 and 3000 pounds per acre,

¹ Original thesis submitted June, 1936. Doctoral thesis number 365.

produced yields significantly greater than those obtained at lower rates of application.

An attempt was made to evaluate the variables causing these differences in yield following applications of rock phosphate. Besides the measurements of reaction, measurements were made of the amounts of exchangeable calcium, available phosphorus and nitrate nitrogen in the soil and of the percentage of calcium in the sweet clover plants.

High exchangeable calcium content of soil was associated with the high lime applications but no significant variability in exchangeable calcium within the lime series or within the unlimed series could be associated with differences in the amounts of phosphates used. This fact, together with the high response of sweet clover to sodium phosphate, which added no calcium to the soil, and the lack of significant differences in the calcium content of sweet clover grown on soils with the different amounts of rock phosphate added, showed that not enough exchangeable calcium was added by rock phosphate to be a factor in sweet clover culture.

Plotting available phosphorus against yields of sweet clover shows an association between available phosphorus content of the soil, yield of sweet clover and the amount of phosphorus applied wherever the same kind of phosphate was used and where the soils did not vary much in reaction. However, there was no significant correlation between yield and the amount of available phosphorus in the soil when the data for the whole experiment were considered.

That phosphorus alone was not the most important factor in yield of sweet clover is shown by the high yields obtained on soils of high pH but of very low phosphorus content and by the low yields of some soils with high available phosphorus content. On the other hand, pH seems to be very important in sweet clover culture. The highest yields were obtained on soils that had the highest pH.

A multiple correlation of yield of sweet clover with pH, exchangeable calcium, nitrate nitrogen and available phosphorus content of the soil and calcium content of the crop showed that pH was the only one of these variables which correlated significantly with yield. This fact, together with the general occurrence of higher yields where there were increases in pH values, indicates that the neutralizing effect of rock phosphate may be responsible in at least a small way for the increases in yield of sweet clover resulting from its use as a fertilizer.

The scope of the work was broadened in a third experiment to determine the effects of five different phosphate carriers on the reaction of three different acid soils as measured by pH. The soils used in this experiment were Grundy silt loam from southern Iowa, Tama silt loam from eastern Iowa and Carrington silt loam from northeastern Iowa. They were selected as being representative of the large areas of acid soils in those sections of the state. Because of the recent interest in the more concentrated phosphates, treble superphosphate, Ammo Phos "A" and ammoniated phosphate were used in comparison with rock phosphate and superphosphate. Three hundred pounds of superphosphate and equivalent amounts of ammoniated phosphate and of Ammo Phos "A" resulted in highly significant increases in the acidity of these three soils as measured by pH. Larger amounts of these materials caused further increases in acidity. Rock phosphate decreased the acidity of these soils to a highly

significant amount as also did treble superphosphate, although not to as great an extent as did the rock phosphate. Larger applications of these materials caused further decreases in acidity.

The effect of the different phosphate fertilizers on soil reaction was found to vary with soil type.

THE DISSIMILATION OF CARBOHYDRATES BY THE COLON-AEROGENES BACTERIA¹

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Although an extensive literature dealing with the dissimilation of carbohydrates by the colon-aerogenes group is already in existence, results as reported are frequently highly contradictory. The present investigation was undertaken with the object of gaining some further insight into the mechanism of carbohydrate dissimilation by that group of organisms.

In attempting to attain that objective, the following methods of attack were used:

1. The periodic analysis of fermentations with the determination of the relationships between substrate and products throughout the course of the fermentation.

2. The determination of the effect of varying conditions upon the ratios of fermentation products.

3. The comparison of the products formed from the dissimilation of different carbohydrates (glucose and xylose).

4. The action of the organisms upon compounds which they normally produce from glucose, both alone and in the presence of a fermentation of the sugar.

The experimental results show no significant differences between the mode of action of typical *Escherichia coli* and the M. R. positive, citrate positive, coli-aerogenes intermediates, upon glucose. Aeration of fermentations of glucose by *Esch. coli* results in the production of detectable quantities of acetylmethylcarbinol, and the accumulation of pyruvic acid as an end-product. Evidence indicates that the accumulated pyruvic acid does not result from the oxidation of lactic acid, but rather from the intervention of oxygen as a hydrogen acceptor preventing the normal reduction of intermediately formed pyruvic acid to lactic acid.

The periodic analysis of fermentations of glucose by *Esch. coli* and *Citrobacter freundii* demonstrates the tendency of those fermentations to change from a predominantly ethyl alcohol-acetic acid producing initial system to a system finally producing chiefly lactic acid. The same fact has been previously noted by Grey (1). The same method of attack shows that the dissimilation of glucose by *Esch. coli* is not a mechanism readily adaptable to explanation by a simple equation or series of equations. The fact that a constant ratio between substrate and products could not be obtained can only be explained as the result of variations in the rate of conversion of glucose to the given products, or to further conversions on these products, or to a combination of the two effects. The data obtained

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by periodic analysis of fermentations and of fermentations of glucose in the presence of added organic acids, indicate that both acetic and succinic acids can undergo further conversions, probably through a system of reactions analogous to the Thunberg-Wieland series (2), and thus play the role of intermediary compounds. Suspensions of *C. freundii* produce pyruvic acid and carbon dioxide from succinic acid under conditions of aeration.

The fermentation of xylose by *Esch. coli* led to the production of lactic acid in approximately the molar equivalent of the xylose fermented. The data indicate that the preliminary attack on the pentose molecule consists of a cleavage into 3- and 2-carbon compounds, the three carbon fraction being subsequently converted to lactic acid. From xylose, much larger quantities of succinic acid were produced than from glucose under the same conditions. The above facts suggest that succinic acid owes its formation to the condensation of some 2-carbon intermediary rather than to the 2- and 4-carbon cleavage of hexose molecules as suggested by Virtanen (4) and by Scheffer (3).

Esch. coli reduces acetylmethylcarbinol to 2,3- butylene glycol in the presence of a fermentation of glucose. Evidence indicates that the reduction involves the same enzyme system and source of hydrogen as those causing the reduction of intermediately formed acetaldehyde to ethyl alcohol.

Although the results obtained under some conditions can be fitted to a reaction scheme such as that proposed by Scheffer (3), results under varied conditions and many data reported in the literature cannot be explained by such relatively simple mechanisms. Evidence available at present is not sufficiently conclusive to justify the acceptance of any one scheme of dissimilation in explanation of the mechanism of the conversion of glucose to the products of its fermentation by *Esch. coli*. Two or more alternatives with equally good experimental basis can be given in explanation of the formation of each end-product and probable intermediary compound. The fermentation appears to be a highly complicated system in which both the rates of formation of the various products from glucose and the rates of their further conversion, are continuously changing.

A periodic study of the fermentation of glucose by *Aerobacter indologenes* shows that acetic acid accumulated during the early phase subsequently undergoes a pronounced decrease, thus displaying the characteristics of an intermediary compound. The production of ethyl alcohol is linear with respect to the sugar fermented, as is also the sum of 1-carbon compounds, i. e., formic acid and carbon dioxide, indicating that the decrease in acetic acid was not the result of its conversion to any of the latter compounds. The facts that 2,3-butylene glycol and acetic acid show a reciprocal relationship and that the sum of one-half the acetic acid with acetylmethylcarbinol and 2,3-butylene glycol gives a linear function, suggest that acetic acid is reduced and condensed to give 2,3-butylene glycol. Substantiation of the above conception is found in the demonstration that acetic acid added to a fermentation of glucose by *A. indologenes* is utilized and that its disappearance is accompanied by an increased yield of 2,3-butylene glycol and the absence of hydrogen in the evolved gas.

The increased yield of 2,3-butylene glycol in the presence of added acetate is approximately equivalent to one-half of the utilized acetic acid.

Acetate alone in a peptone medium was not reduced by *A. indologenes* in the presence of an excess of hydrogen. When an excess of hydrogen was supplied to a glucose-acetate fermentation, the quantity of acetic acid reduced corresponded with that reduced when extra hydrogen was not supplied. Under the conditions used, then, *A. aerogenes* does not activate molecular hydrogen for the reduction of acetic acid. Some more active form, possibly atomic hydrogen or an active hydrogen donator, must be necessary for that reduction.

The fermentation of xylose by *A. indologenes* leads to products comparable with those produced from glucose. From xylose, however, appreciable quantities of succinic acid were produced while that acid was not found among the products of the fermentation of glucose. Results suggest that succinic acid may play an intermediary role in the fermentation of xylose by *A. indologenes*.

Succinic acid is utilized by *A. indologenes* in the presence of glucose.

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A METHOD OF QUANTITATIVE CHEMICAL ANALYSIS USING A PHOTON COUNTER¹

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The successful application of spectrum analysis to quantitative determinations depends upon an accurate measurement of the intensity of the characteristic spectrum line or lines of the element considered. In the work on this problem a photon tube was used to measure the intensity of the 2347A beryllium line as different amounts of the latter element were introduced into an arc between two graphite electrodes. The light from this arc was passed through a Gaertner single prism, constant deviation type of quartz monochromator.

Several types of photon tubes were constructed similar to those described by Rajewsky (1) and Locher (2). The tube used in most of the work is similar to that of Locher. The Pyrex tube with a quartz window and a silver cathode is filled with 8 cm. of helium gas. A metal case surrounding the tube prevents stray light from entering.

The potential required for the field of the counter tube is furnished by a direct current power pack similar to that described by Schmitt (3). The power transformer has a maximum potential of 2,000 volts across the secondary. Half-wave rectification is obtained by an 866 mercury vapor rectifier. The filter system consists of two 300 henry, 15 ma. chokes and three 1 m.f.d. condensers. All transformers and condensers are insulated for 2,000 volts. Voltage fluctuations are reduced by use of a '57 tube as a voltage regulator. Fluctuations in voltage are negligible even for line voltage variations of 10-15 volts. The output voltage can be varied from zero to nearly 2,000 volts.

The impulses from the photon tube are very small, making it necessary to have a sensitive amplifier to detect and record them. Several types of high gain amplifiers were tried. The one finally adopted is a modification of that used by Locher (4). The amplifier consists of a two-stage resistance-coupled circuit. A high gain 257 tube was used in the first stage and a 2A5 in the second stage. The latter was used to drive the speaker and the counting mechanism. The photon counter is connected to the amplifier by a 10 μ .f.d. condenser. A 300 megohm resistor is connected in series with the photon tube and the high voltage power pack. A filter system for the grid bias resistor is found necessary to reduce "feed back" into the 257 tube. The screen and plate circuits are also well filtered. The power supply for the amplifier, including transformers and filter system, is removed to a distance to prevent the amplifier from being affected by induced currents and mechanical vibrations. All leads from the high voltage source, photon tube and power pack are shielded and the shield grounded. By having the speaker connected in the plate circuit of the 2A5 the impulses passed on to the counting mechanism may be noted.

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Since the impulses from the amplifier are at times more rapid than can be resolved by the mechanical recorder, it is necessary to have some means of reducing the number of counts per unit of time to such a value as can be recorded. The Wynne-Williams (5) "Scale of Two" thyatron counting circuit offers such a mechanism, since the impulses entering the counter are reduced by a "scale of two" for each pair of thyatrons employed. In this counting mechanism, a modification of the Wynne-Williams circuit is used. Four thyatrons are used, thus requiring four impulses from the amplifier to complete the cycle in the last pair of thyatrons. The mechanical recorder used is a modified form of that suggested by Van den Akker (6) for counting impulses from a Geiger-Müller tube. It consists of two permanent magnets between whose poles a small vibrator of transformer iron is placed. The vibrator is prevented from touching the poles by two small brass stops. Around the vibrator is placed a helix composed of two separate coils of wire. These coils are wound simultaneously and contain the same number of turns. Each of these coils is connected in the anode circuit of one of the last pair of thyatrons. As first one and then the other of the last pair of thyatrons is lighted the vibrator is driven from one pole to the other of the permanent magnet. The movements of the vibrator are recorded by a stop watch. The balance wheel of the watch is removed and the end of the vibrator connected to the escapement lever of the watch.

By means of a capillary pipette, a certain volume of a solution containing a known concentration of beryllium per cubic centimeter is introduced into a specially prepared cup in the lower graphite electrode. After evaporation, the electrode is placed in its holder and adjusted to a specified position by an optical focusing arrangement. Adjustment of the voltage across the photon tube to a standard value is accomplished by checking the counting rate produced by a standard radium sample. The arc is struck by using a graphite rod. Simultaneously the watch used for timing is started. The counting mechanism will start as soon as light strikes the photon tube. The period of excitation can be determined in two ways. One may either select a certain time, for example 2 minutes, for all excitations, or may continue until the entire electrode cup is burnt. With the type and size of electrodes used these two methods give quite similar results. The time required for complete burning is about two and one-half minutes. The usual procedure is to run several blank electrodes both before and after those containing the samples. The blank electrodes are prepared in exactly the same manner as those containing the beryllium samples. Thus one obtains a count from the blanks which is characteristic for all the electrodes of that type. The actual count registered by the counting mechanism is corrected by subtracting the count obtained for the blank electrodes. This corrected count is plotted against milligrams of beryllium present. The graphs obtained represent the variation of the number of counts with the number of milligrams of beryllium present upon the electrode.

Eight different test runs were made and the results plotted. The results of one of these runs is shown in the following table.

Run No. 3
Time—2 Minutes

Electrode Type A
Treatment—Total Lacquer

Milligrams Be	Counts		Counts		Counts	
	Actual	Correct	Actual	Correct	Actual	Correct
0.10	225	118	223	116	231	124
0.05	206	99	209	102	lost	lost
0.03	178	71	190	83	190	83
0.01	146	39	141	34	142	35
Blank	107	107	107	107	107	107

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THE CHEMICAL TRANSFORMATION OF ALIPHATIC ACIDS IN THE COURSE OF THE BUTYL-ACETONIC FERMENTATION¹

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The chemism involved in the butyl-acetonic fermentation has been the subject of considerable research and speculation. The mechanism for the transformation of carbohydrates to the end products must necessarily be very flexible to account for the variation in ratios of the different products formed under varying conditions, the constancy of the same end products with a number of utilizable substrates, and the shifting nature of the fermentation in its different phases. There are several general methods for testing the probability of a reaction scheme in a fermentation process. In one procedure an isolation of the intermediates is attempted. However, the isolation of a given compound does not offer conclusive evidence that it is an intermediate unless it can be definitely established that the compound is a precursor of one or more of the end products. Another approach concerns the addition of non-proliferating cells or cell preparations to solutions of possible intermediates. A third procedure is the addition of the supposed intermediates to an active culture of the organism. The value of transfusing a postulated intermediate to an active fermentation as a means of gaining an insight into a fermentation mechanism is based upon the premise that the substance is fermentable and is subject to the same chain of reactions that occurs in the normal fermentation. It should also be kept in mind that the amount of a transfused chemical which the organism is able to utilize is an important consideration. An intermediate, when added to an active culture, should be converted quickly, completely, and in large amounts to its purported end products.

The purpose of the present study was to obtain further information concerning the chemical changes taking place in the butyl-acetonic fermentation. The line of attack pursued consisted in the addition of certain aliphatic acids to an actively growing culture of the butyl organism with subsequent analysis of the end products of the fermentation to determine the fermentability of the added acids and their course of chemical transformations.

As different cultural conditions bring about changes in the chemical processes involved, a first and most important caution was to guard against undue alterations from the normal course of fermentation. This led to the development of a procedure which permitted transfusion of various compounds to corn mash media with minimum deviations from controls. The organisms were grown in five per cent corn mash and in each experiment the initial inoculation was made from a stock spore culture kept on sea sand.

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From the experimental data it was found that *n*-butyric acid was transformed into *n*-butanol and to a somewhat lesser extent into acetone. The production of carbon dioxide was increased and that of hydrogen decreased.

Acetic acid was converted almost entirely into acetone.

n-Butyric-acetic acid mixtures gave optimum yields of solvents when the ratio was 2:1. This mixture also resulted in yields of all solvents; the major transformation was to *n*-butanol with least conversion to ethanol.

Formic acid, even when added to an extent of less than four-tenths of a gram per liter of five per cent corn mash, proved toxic to the organism.

The transfusion of *n*-propionic acid resulted in the formation of a small amount of *n*-propanol together with acetone.

Evidence was obtained indicating that isobutyric acid was partially reduced to isobutyl alcohol.

The pH levels of a series of fermentations were varied from 3.8 to 5.3 by the addition of HCl or NaOH. Except for the fermentation carried out at a pH of 3.8, the solvent yields were fairly uniform although there was a slight tendency for increased acetone and *n*-butanol production where the pH was slightly more acid than that of the control.

The highest tolerance exhibited by the organism toward the transfused acids was for *n*-butyric acid, which could be added to an extent of three and one-half grams per liter of five per cent corn mash.

The results of this investigation tend to show that the butyl organism may produce varying quantities of one, two, or all three of the different solvents from the various transfused compounds. From this, we have the choice of several conclusions:

1. The transfused products may be direct intermediates in the fermentation, and are precursors to the given solvents as postulated in the different proposed mechanisms.

2. The transfused assumed intermediates may undergo the transformations assigned to them in the various mechanisms, but the course of the fermentation may be so altered that apparent conversion to other solvents occurs. This explanation would account for the apparent transformation of *n*-butyric acid into acetone.

3. The transfused substances may be regarded simply as fermentable substrates, and the solvents derived from a series of complex reactions involving the synthesis of *n*-butanol, acetone, and ethanol.

While these acids are fermentable, the limited tolerance displayed by the organism toward them does not seem to justify the conclusion that they are the sole intermediates to acetone and *n*-butanol.

GASTRIC DIGESTION OF SOYBEAN FLOUR WHEN USED AS A SUBSTITUTE FOR COWS' MILK IN FEEDING DAIRY CALVES¹

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One of the most serious problems with which the dairy farmer is concerned is the economical rearing of calves. A vast amount of work has been done to produce calf feeds which may displace milk in the calf ration, many stations having been outstanding in their interest in calf meals and gruels. The problem has usually been attacked either (1) by attempting the use of milk products such as dried and semi-dried skim-milk and buttermilk, or (2) by using cereals, packinghouse by-products, sugar, starch, etc., in the making of meals or gruels.

The use of soybean products in the rations of mature animals, and even for human consumption, has been quite extensive. Their use for human consumption among the poorer classes in the Orient is probably more common than the use of wheat. The Chinese have made rather extensive use of "milk" made from the soybeans, in human infant feeding. This suggested the use of soybean "milk" to replace cow's milk in feeding dairy calves. Accordingly a series of experiments was inaugurated to determine the rate at which soybean flour, fed as a gruel, passes from the abomasum of the calf. The secretion of gastric juice, which was used as a means of determining the rate of passage of the soybean flour from the stomach, was measured by (1) determining the volume of pure gastric juice secreted in Pavlov pouches, and (2) by determining the acidity of the stomach contents by use of rumen fistulae.

Three series of trials were conducted:

1. A series of twelve-hour trials using a test meal of one liter of soybean milk or whole cow's milk, fed after a fasting period of twenty-four hours.
2. A continuous trial of fourteen days, comparing soybean milk and whole cows' milk, with regular feedings of three to four pounds at eight-hour intervals, with seven days on soybean milk and seven days on cows' milk.
3. A series of sixteen-hour trials comparing test meals of one liter of skimmed cows' milk to one-half liter of "fortified soybean milk" (described below), fed after a fasting period of twelve hours, preceded by twelve hours on oatmeal gruel, in which (a) volume of gastric secretion was determined, and (b) free and total acidity of gastric contents were determined by titration, in calves with rumen fistulae.

The soybean milk used in series 1 and 2 was a mixture of 1 part soybean flour in 9 parts warm water. The "fortified soybean milk" was made by mixing fresh skim milk with soybean flour so that one-third of the dry matter of the mixture came from skim milk and two-thirds from soybean

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flour. This mixture contained 20 per cent dry matter. Eight cubic centimeters of a 40 per cent solution of calcium chloride per liter was also added to aid in coagulation. The whole milk used had a fat content ranging from 2.69 to 3 per cent and a curd tension of 80 to 95 grams. Skimmilk used as a check in the sixteen-hour trials, as well as a solvent for the soybean flour, came from the same cow.

The gastric acidity determinations were made by titration with N/100 NaOH, using Töpfer's reagent and phenolphthalein as indicators, for free and total acidity, respectively.

The results of the twelve-hour series in which four calves were used showed that, during the twelve-hour period, the volume of secretion on soybean milk was 11.87 per cent higher than that on whole cow's milk. During the first six hours of the twelve-hour period the secretion on soybean milk was 21.41 per cent higher than that on whole cow's milk, while for the second six hours the secretions caused by the two feeds were virtually equal. Moreover, the maximum secretion (largest volume of juice secreted in one half-hour period) was in every case larger when the soybean ration was fed, this increase averaging 39.35 per cent. These results indicate that the soybean diet functioned as a stronger secretagogue in the stomach, and that it left the stomach more rapidly than whole cows' milk.

In the continuous series the total volume of gastric secretion was slightly less when soybean gruel was fed than when whole milk was used. It is thought this reversal of results was due in part at least to the fact that in this series the calves were full-fed and that the volume of juice secreted per unit of protein material was less under these conditions. Moreover previous experiments show that much of the test meal spills into the rumen instead of all of it going directly into the abomasum. This may have affected the secretagogic capacity of the soybean material.

The results of the sixteen-hour trials show that the "fortified soybean milk" behaved somewhat like the skimmilk with which it was compared, for the volumes of secretion were near one another, although in all cases the secretion on soybean gruel was slightly larger. The volume of juice produced during the last half of the experimental period, when soybean milk was fed also increased 3.28 per cent over that when the skimmilk was fed, which indicated that the soybean material remained in the stomach for practically the same length of time as did the skimmilk. The secretion during the first half of the period was 7.71 per cent larger when soybean milk was fed than with skimmilk.

The results of determinations of acidity of the stomach contents when soybean milk and skimmilk were fed, showed that in all cases the soybean milk evoked on the average 12.58 per cent greater acidity in gastric contents than did skimmilk. On the other hand, the free acid was less by 19.2 per cent when the soybean milk was fed, than with skimmilk. Free acid appeared with the soybean diet four hours after feeding and with the skimmilk, two hours after feeding.

These results indicate that: Assuming that the volume of gastric secretion is in direct proportion with gastric digestion, then soybean flour, fed as in these trials, is digested in the calf's stomach at a slightly more rapid rate than either whole or skimmed cows' milk.

STUDIES ON THE GROWTH AND REPRODUCTION IN THE RAT¹

(1) THE VALUE OF DIFFERENT COD LIVER OILS FOR REPRODUCTION

(2) THE VALUE OF CERTAIN INDIVIDUAL FOODS AS SOURCES OF VITAMINS B AND G FOR GROWTH, REPRODUCTION AND LACTATION

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The vitamin E content of eight different brands of cod liver oil was investigated. The basis for comparison was the number of young born to females on diets in which these cod liver oils furnished vitamins A, D and E. No study was made concerning the possible oxidative destruction of the vitamins. One oil was shipped and stored in an iron container. This oil was the poorest from the standpoint of reproduction and its oxidative destruction was decidedly possible.

Diets containing cod liver oil No. I gave decidedly better reproduction than diets containing other brands of oil. No. II and No. III oils were of about equal value for reproduction, but were inferior to No. I. The No. IV brand was inferior to the three oils mentioned when comparisons were based on reproduction records. The oils known as No. V, No. VI and No. VII were of about equal value for reproduction, but were inferior to all other oils studied with the exception of No. VIII cod liver oil. The No. VIII oil permitted the least reproduction of any of the oils studied. It is significant that this oil was shipped in an iron container and that oxides of iron were present in the oil when this oil was withdrawn for use. The oxides of iron might have contributed to some oxidative destruction of the vitamins originally present in the oil.

Reproduction on diets containing five per cent of No. I cod liver oil as the sole source of vitamins A, D and E was compared with reproduction on diets containing five per cent of butterfat as the sole source of these vitamins. The diets containing the oil were uniformly better than those containing the butter, indicating a higher content of vitamin E in the oil.

Certain females on diets containing the different brands of cod liver oil were permitted to raise their young as a measure of their lactation ability. Diets containing No. I oil were the best for lactation. This superiority was shown by the lower mortality and the higher weaning weights of the young.

Diets containing No. I cod liver oil as the sole source of vitamins A, D and E were studied to determine whether yeast, wheat embryo or ether extracted wheat embryo was the best source of vitamins B and G for reproduction and lactation. Diets containing No. I oil and extracted wheat embryo were decidedly superior to diets containing either of the other sources of vitamins B and G. The yeast and unextracted wheat embryo were of about equal value for reproduction and lactation.

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Observed differences in reproduction were small throughout the study of the various cod liver oils. In every case the result on the experimental diets were compared with results using the diet employed for our stock colony. This was in the nature of a control over the experimental work. It soon became apparent that duplicate experiments run during different seasons of the same year or run during the same season of different years would not show identical results. This led to a study of reproduction and lactation in our stock colony. A study of these normal animals on a normal diet revealed the fact that variations in reproduction and lactation were larger than variations which had been considered significant in some previous experimental work. The study was continued for twenty-eight months and the number of young per female per month as well as the mortality of the young during each month showed the young per female in December, 1930, to be 0.22 while the young per female in June, 1930, appeared as 2.06. The mortality of the young in July of 1930 was 3.6 per cent while the mortality in December of 1930 was 64.3 per cent. These were the outside limits of the variations during the time of the investigation.

Purified diets, adequate in all respects except in vitamins B and G, were supplemented with various amounts of the diet used in our stock colony. Reproduction and lactation improved progressively with increases in the amount of the growing ration. This growing ration itself was improved by the addition of butterfat and was also improved by the addition of fish meal.

Reproduction and lactation were studied, using diets containing wheat as the sole source of vitamins B and G. These diets were further supplemented with various components of our growing ration. The components studied were tankage, buttermilk powder, linseed oilmeal, fish meal and alfalfa. The wheat diets were improved by the addition of every component with the exception of tankage. Buttermilk powder and fish meal were especially beneficial.

The vitamin B and G content of wheat, alfalfa, tankage, buttermilk powder, fish meal, linseed oilmeal and cottonseed meal was investigated in a series of lactation studies. Females from the stock colony were placed on the experimental diets at the time of parturition. These diets were adequate in all respects except in vitamins B and G and were supplemented with different percentages of each of the above components of our growing ration. The percentages used were ten, twenty-five, forty and sixty. Lactation was below normal on all of these diets studied. The twenty-five per cent level was selected for further study.

Females from the stock colony were placed on the diet which contained twenty-five per cent of the selected component as soon as the young were born. These females were each given certain vitamin supplements. The nature of these supplements and the amounts fed to each female daily are discussed below. The first supplement consisted of three-tenths of a gram of vitamin B adsorbate on fuller's earth. The second supplement consisted of one and one-half grams of vitamin G preparation from hog liver. The third supplement consisted of the vitamin B concentrate plus the vitamin G preparation from hog liver. The fourth supplement consisted of the vitamin B concentrate plus two and four-tenths grams of

autoclaved yeast. Success in lactation was judged on the basis of the mortality of the young and the weaning weights of the young.

These experiments indicated that the components deficient in vitamin B were tankage, buttermilk powder, linseed oilmeal, fish meal and possibly cottonseed meal. The components deficient in vitamin G were wheat, tankage, buttermilk powder, linseed oilmeal and cottonseed meal. Diets containing any one of these components, supplemented by vitamin B plus vitamin G, permitted lactation equivalent to the lactation on our growing ration. The vitamin G preparation from hog liver appeared to be better for lactation than autoclaved yeast.

DISSIMILATION OF CARBYHYDRATES BY BACTERIA OF THE GENUS AEROBACILLUS¹

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This investigation was undertaken with a two-fold objective: (1) to study the dissimilation of glucose and xylan and its derivative, xylose, by *Aerobacillus polymyxa* and *A. acetoethylicus*; (2) to investigate the formation and mutual relationships of acetylmethylcarbinol and 2,3-butylene glycol in fermentations.

Xylose is the hydrolytic product of xylan, which is an important component of such agricultural wastes as straws, oat hulls and cornstalks and cobs. A preliminary experiment showed that 43 to 63 per cent of xylan and 51 to 67 per cent of the pentosan content of corn cobs were fermented by *A. polymyxa*. Xylose in 1 per cent solutions was completely fermented. The acid hydrolysis of the xylan in agricultural wastes and the subsequent fermentation of the crude hydrolytic product may be of importance both to agriculture and to industry.

The medium used in these studies on the dissimilation of xylose and glucose consisted of 0.5 per cent CaCO_3 , 0.2 per cent K_2HPO_4 , 0.5 per cent peptone, 0.5 per cent yeast extract and 1 or 2 per cent carbohydrate. The addition of the yeast extract markedly increased the rate of fermentation, reducing the time for completion from 6 or 7 days to as low as 48 hours.

The products of the fermentation of xylose were found to be the same as those from glucose and in the same proportions. The fermentation of these sugars by *A. polymyxa* results in carbon dioxide, hydrogen, ethyl alcohol, acetylmethylcarbinol, 2,3-butylene glycol, and acetic, formic, lactic and succinic acids. *A. acetoethylicus* forms acetone and all of the other products mentioned except acetylmethylcarbinol (sometimes in traces) and 2,3-butylene glycol.

The fermentation of xylose by *A. acetoethylicus* in a medium containing NaHCO_3 results in much more formic and acetic acids and less acetone than in a similar medium containing CaCO_3 . In one experiment with NaHCO_3 26.7 per cent of the fermented xylose was represented by formic acid, 30.6 per cent by acetic acid and 0.1 per cent by acetone, while a similar experiment with CaCO_3 resulted in 4.6 per cent of xylose as formic acid, no acetic acid and 11.1 per cent acetone. *A. polymyxa* forms more formic and acetic acids and less 2,3 butylene glycol from xylose with NaHCO_3 than when CaCO_3 is used. The difference in yields is probably the result of the differences in the pH of the media. CaCO_3 buffers the medium at a pH of 5.8 to 5.9 and NaHCO_3 at approximately 6.5 to 6.6. Osburn (1934) found that free acetic acid exists only below pH 6.3 in solutions buffered with $\text{K}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ mixtures. Above pH 6.3, the

¹ Original thesis submitted August, 1935. Doctoral thesis number 346.

salt of the acid is present in the medium and it fails to undergo further reaction. The same is likely true for formic acid.

The addition of acetic acid to a CaCO_3 buffered glucose containing medium which is fermented by *A. Polymyxa* results in an increased yield of 2,3-butylene glycol plus acetylmethylcarbinol and a decreased yield of hydrogen. Since less acetic acid is present in the fermented liquor than what was added, some was converted to acetylmethylcarbinol and 2,3-butylene glycol. Probably the acetic acid was reduced to acetaldehyde which was then condensed to acetylmethylcarbinol and the latter reduced to the glycol. The addition of acetic acid to a fermentation of glucose by *A. acetoethylicus* increases the acetone and ethyl alcohol and decreases the hydrogen produced. Thus it appears that acetic acid serves both as an intermediary and as an end product in fermentations by *A. polymyxa* and *A. acetoethylicus*.

The evidence that acetaldehyde is an intermediary in the fermentations of glucose by *A. polymyxa* is two-fold: (1) Added acetaldehyde increases the yield of ethyl alcohol, acetylmethylcarbinol, and 2,3-butylene glycol, and (2) the aldehyde was fixed in normal fermentations of glucose by the use of both CaSO_3 and NaHSO_3 . The melting point of the dimedon derivative was 138°C . The acetaldehyde dimedon derivative melted at 139°C . and the mixed melting point was 138°C .

Acetylmethylcarbinol ($\text{CH}_3 \cdot \text{CO} \cdot \text{CHOH} \cdot \text{CH}_3$) and 2,3-butylene glycol ($\text{CH}_3 \cdot \text{CHOH} \cdot \text{CHOH} \cdot \text{CH}_3$) are products of the fermentation of glucose and xylose by *A. polymyxa*. An investigation of the production and mutual relationships of these two compounds was extended from *A. polymyxa* to citric acid fermenting streptococci and the butyl-acetone group of microorganisms.

Little acetylmethylcarbinol accumulates during the fermentation of glucose by *A. polymyxa* even when oxygen is bubbled through the culture. 2, 3-Butylene glycol is produced rapidly and added acetylmethylcarbinol is reduced to the glycol when considerable sugar is present and the oxidation-reduction potential is low. When all the sugar has been fermented and the redox potential has risen considerably, 2, 3-butylene glycol will donate hydrogen to oxygen with the formation of acetylmethylcarbinol. Thus, either compound may serve as the precursor of the other in fermentations.

After a maximum yield of acetylmethylcarbinol plus diacetyl ($\text{CH}_3 \cdot \text{CO} \cdot \text{CO} \cdot \text{CH}_3$) has been obtained in butter cultures, continued holding of the cultures frequently results in a decrease of these substances. The citric acid fermenting streptococci in the cultures apparently are responsible for the decrease. Studies were made to determine the fate of the carbinol and diacetyl in various cultures of these organisms. When added to a tomato bouillon or milk culture of one of the citric acid fermenting streptococci, acetylmethylcarbinol is partially reduced to the corresponding glycol. The method employed was to inoculate the medium and then incubate for 24 or 48 hours to obtain good growth. Acetylmethylcarbinol, or diacetyl, was then added and determinations for the carbinol or diacetyl and 2, 3-butylene glycol were made at once and after various periods of holding. Typical results are given. The molarity of acetylmethylcarbinol in a tomato bouillon culture of organism 29 was reduced from a molarity of 0.0052 to 0.0003 in 72 hours. During this time

the molarity of 2, 3-butylene glycol increased from 0.0006 to 0.0058. In a tomato bouillon culture of organism 49, added diacetyl was reduced from a molarity of 0.0025 to 0.0001 in 24 hours, while the molarity of 2, 3-butylene glycol increased from 0.0010 to 0.0031. The reduction of the carbinol in a tomato bouillon culture of one of the citric acid fermenting streptococci is hindered by reducing the pH to 3.8 by the addition of sulphuric acid. The addition of 0.5 ml. of 30 per cent H_2O_2 per liter of skim milk culture or 1.5 ml. of either acetaldehyde or propionaldehyde per 1200 ml. of culture markedly inhibited the reduction of the carbinol.

Clostridium acetobutylicum normally produces small quantities of acetylmethylcarbinol but is unable to reduce added carbinol to 2,3-butylicum glycol during a fermentation of glucose. *Cl. pectinovorum*, *Cl. butylicum* and *Cl. pasteurianum* do not form the carbinol in normal fermentations but are able to completely reduce added carbinol to the glycol. *A. polymyxa* and the citric acid fermenting streptococci are able both to form the carbinol and to reduce it to 2,3-butylene glycol. Since *Cl. acetobutylicum* forms butyl and ethyl alcohols by the reduction of the corresponding acids, the failure to reduce acetylmethylcarbinol suggests that a different dehydrogenase is required for the carbinol-glycol change than for the reduction of the acids.

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BACTERIOLOGICAL STUDIES ON SOME DEFECTS OF CREAM CHEESE SPREADS¹

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Within recent years the manufacture of cream cheese spreads has developed into an important branch of the dairy manufacturing industry. In general, cream cheese spreads consist of cream cheese to which various products are added to secure a variety of flavors; the spreads are heated to a relatively high temperature and placed, while hot, in the final container, which is often a vacuum sealed glass. In cream cheese spreads, like most other dairy products, certain defects have appeared which require bacteriological study in order to prevent recurrence and resulting financial loss to the manufacturer.

No work has been reported on the bacteriology of cream cheese spreads. A number of investigators have found anaerobic organisms present in various dairy products. Csiszar, in studying the bacteriological defects of process cheese, found that the organisms responsible for spoilage were *Bacillus sporogenes*, *Bacillus putrificus* and *Bacillus saccharobutyricus*. He found that these organisms could not be killed in process cheese by lowering the pH or adding salt or any other preservatives which he studied, without lowering the quality of the cheese.

STUDIES ON GAS PRODUCTION IN CREAM CHEESE SPREADS

The outbreak of gas formation in cream cheese spreads which was responsible for this study occurred in the spring and summer of 1934. Gassiness was observed to a limited extent in several varieties of spreads but the defect was particularly noticeable in Roquefort type spread.

Only a small percentage of the jars in a batch developed gas. Under ordinary marketing conditions the cheese spreads were held at room temperature and at times remained normal for 2 or 3 months and then suddenly developed gas. When the spreads were held at 37° C. gassiness usually developed in 5 to 10 days if it developed at all. The gassy spreads had no off flavor or odor and appeared normal in every way except that gas was produced in the cheese. No typical gas holes were apparent, but the spread, inside the glass jar, would break and the upper portion would be forced from the lower portion with a clean break. The defect varied from only a few breaks in the cheese to the condition where approximately half of the spread was pushed out of the jar.

The organism responsible for the outbreak of gas formation was found to be a heat resistant anaerobic organism. The organism was very unusual in its growth requirements, since it could be grown only in peptone-litmus milk, in litmus milk to which a small amount of Roquefort type cheese had been added and in Roquefort type cheese emulsion.

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Nineteen cultures of the gas producing organism were isolated; 12 came from 12 different lots of defective Roquefort type cream cheese spread, 5 from domestic blue cheese and 1 each from Danish bleu and French bleu.

The gas producing organism was found to be very heat resistant in peptone-litmus milk at high pH values, but as the pH was lowered the heat resistance also was lowered. The addition of salt in concentrations over 2 per cent decreased the heat resistance of the organism at a given pH. Since Roquefort type cheese contains 4 to 5 per cent salt, the addition of enough acid to lower the pH to about 5.40, together with holding the cheese at 85° C. for 20 minutes, should enable the manufacturers to make Roquefort type cheese spread in which gas production does not occur, even though the organism is known to be present.

Since the organism was so heat resistant, it was placed in the genus *Clostridium*, although spores were never observed. The name *Clostridium peptophilum* is proposed. A complete description of the organism could not be prepared since it grew in only a few media.

STUDIES ON LIQUEFACTION IN CREAM CHEESE SPREADS

About the time the outbreak of gassiness occurred, the same plant began having trouble with liquefaction in a few varieties of cream cheese spreads, especially the Roquefort and pineapple spreads. This defect developed in about 3 to 4 weeks at room temperature and in about 5 to 10 days at 37° C. Only a small percentage of the jars in each batch showed liquefaction. The defect occurred in all degrees, ranging from only a small amount of liquid on the surface to the condition where approximately half of the spread in the jar was liquefied. When liquefaction was extensive the liquid collected around the outside of the cheese spread and was translucent. In a few cases enough gas was produced to release the vacuum seal. When the lid was removed a very pronounced putrid odor was noted.

Liquefaction in cream cheese spreads was found to be due to the action of a heat resistant spore bearing organism that was identified as *Clostridium sporogenes*.

Clostridium sporogenes was not killed by heating at 95° C. for 20 minutes in litmus milk with a pH of 5.21 and a salt concentration of 10 per cent. It was possible to inhibit the digestion of the milk by combinations of high salt and low pH values, but in all cases the salt concentrations and pH values which inhibited digestion could not be used commercially without ruining the quality of the spread.

I. THE RELATIVE AROMATICITY OF FURAN

II. HEAVY HYDROGEN IN SOME NATURALLY OCCURRING ORGANIC COMPOUNDS AND MIXTURES

I. THE RELATIVE AROMATICITY OF FURAN

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Furan has previously been shown to have pronounced aromatic properties by Gilman, *et al.* These investigators used the acid cleavage of furyl-aryl-lead compounds (1a), nitration of furyl aryl ketones (1b), Friedel-Crafts reactions on furan compounds (1c), and the metalation of furan compounds (1d).

In the present work competitive substitutions have been applied to compounds containing aryl and furyl nuclei.

The nitration of phenyl 2-furoate to yield phenyl 5-nitro-2-furoate (m. 121-1.5°) was perhaps the most significant evidence found for the pronounced aromaticity of furan. This fact will be more readily grasped when one considers that nitration of phenyl benzoate results in 4-nitro-phenyl benzoate (2). Nitration of 3-methoxyphenyl 2-furoate (b. 179°/10 mm.) resulted in a mixture of 3-methoxy-6-nitrophenol and its furoic ester m. 106-6.5°. Nitration of diphenyl dehydromucate (m. 138.5°) gave picric acid only, while nitration of 2-naphthyl 2-furoate (m. 121-2°) gave a mixture which upon oxidation yielded 2-furoic acid. Acetylation of phenyl 2-furoate by means of stannic chloride and acetic anhydride in benzene solution was unsuccessful.

Bromination, nitration and mercuration of 2-furylphenylmethane and 1-(2)furyl-2-phenylethylene (m. 53-4°), prepared by decarboxylation of β -(2)furyl- α -phenylacrylic acid, gave negative results, only tars and decomposition products being isolated. An excellent derivative of 2-furylphenylmethane was found in Δ^4 -3-benzyl-3,6-endoxytetrahydrophthalic anhydride (m. 102-2.5°), prepared by the action of maleic anhydride on the furylphenylmethane.

The sulfonation of 2-benzoylfuran gave 5-benzoylfuran 2-sulfonic acid (isolated as the barium salt). Bromination of 2-benzoylfuran gave 5-bromo-2-benzoylfuran (m. 37-7.5°; oxime, m. 139-40°), while bromination of 2-(*p*)anisoylfuran gave an addition product (m. 131-2°) which lost hydrogen bromide to yield 5-bromo-2-(*p*)anisoylfuran (m. 73-4°). Mercuration of 2-benzoylfuran resulted in highly-mercurated products in which the amount of mercury corresponded to no calculated values. Nitration of 2-(*p*)toluylfuran (b. 185.7°/18 mm.), prepared from toluene, 2-furoyl chloride and aluminum chloride, resulted in 5-nitro-2-(*p*))toluylfuran (m. 122-3°), and nitration of 2-(*p*)-anisoylfuran gave 5-nitro-2-(4)methoxy(3)nitrobenzoylfuran (m. 123-4°) even when reasonable pre-

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cautions to effect mononitration were taken. 5-Nitro-2-(*p*)anisoylfuran (m. 126-7°) was prepared by replacing the bromine in 5-bromo-2-(*p*)anisoylfuran by means of nitrogen trioxide. Nitration of this compound gave the former dinitro ketone.

Attempts to prepare 2-(2)furoylpyridine by the action of 2-furylmagnesium iodide on 2-cyanopyridine were failures.

Nitration of 5-nitro-2-benzoylfuran was unsuccessful, and the nitration of 2,5-dibenzoylfuran gave the two compounds reported by Phelps and Hale (3). The compound melting at 193° was most likely 2,5-di(*m*)-nitrobenzoyl-3-nitrofuran, but the other compound (m. 129-30°) may have been a ring scission product. Analyses of the latter product were correct for a mononitrated compound, but oxidation gave only an acid (m. 132-3°) whose identity is as yet obscure. Mercuration of 2,5-dibenzoylfuran was unsuccessful.

Furonitrile apparently possesses some unorthodox properties since nitration to yield 5-nitro-2-furonitrile (m. 67.8°) had to be forced, while benzonitrile nitrates readily under mild conditions (4) and other negatively substituted furans nitrate (5) much easier than do their benzene analogs.

Several Friedel-Crafts reactions were performed using stannic chloride, acetic anhydride and the furan compound in toluene solution. 2,5-Dimethylfuran and ethyl 2-furoate acetylated readily with formation of only traces of *p*-methylacetophenone. Methyl 5-bromo-2-furoate gave *p*-methylacetophenone and recovered ester as the only products, while in benzene solution no acetophenone was formed.

SUMMARY

Nitration, bromination and sulfonation have been successfully applied to the relative aromaticity of furan. Furan is more aromatic than benzene, toluene and anisole and would probably lie close to anisole in a series of aromaticity on the basis of nuclear substitution.

Some indirect evidence concerning the furan *beta*-carbon has been presented.

II. HEAVY HYDROGEN IN SOME NATURALLY OCCURRING ORGANIC COMPOUNDS AND MIXTURES

The natural abundance of deuterium has received a good deal of attention in the past few years. Unfortunately, several objections have arisen (6) which render worthless most, if not all, of the accumulated data. These criticisms revolve about the fact that previous investigators have neglected the difference between the atomic weights of commercial oxygen and that of normal oxygen, and the difference between the atomic weights of oxygen in the air and oxygen in water which amounts to six parts per million difference in the densities of water prepared from these two oxygens, the air-oxygen water being the heavier (6).

The combustions of fungi yeast, etc., offer some difficulty and this thesis presents a simple method of effecting adequate combustion of these substances.

Previously unpublished results of the determination of heavy water in beef tissues were also reported in this thesis. The particular tissues in-

vestigated were the hide, kidney, prostate, spinal cord, pancreas, thyroid, muscle, ovary, thymus, heart, aorta, testis, marrow, spleen, lung, brain, liver, liver water, blood (defibrinated), blood water, blood fibrin, haemoglobin, blood serum and tallow of adult cattle. This work was performed under the direction of Dr. Henry Gilman in collaboration with Dr. H. L. Keil, Dr. A. W. Ralston, Mr. V. Conquest, Dr. W. H. Jennings, Dr. W. E. Catlin and Mr. M. T. Kelley.

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AN INVESTIGATION OF TYPES OR STRAINS OF THE MOSAIC VIRUS OF SUGARCANE IN LOUISIANA¹

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The virus which causes the mosaic disease of sugarcane and related grasses has been divided into four strains based upon the symptoms produced on two different host varieties, C. P. 28/60 and Louisiana Purple. Strain 1 produces a pattern characterized by very mild chlorosis and no necrosis or stunting on C. P. 28/60; strain 2 produces severe and general chlorosis, varying amounts of necrosis, and pronounced stunting on the same variety; while strains 3 and 4 produce identical symptoms of severe chlorosis in the form of streaks and a necrotic condition that may or may not severely blight and sometimes even kill the growing point. Strains 1, 2 and 4 produce only ordinary, rather mild, symptoms of Louisiana Purple and several other commercial varieties, while strain 3 has approximately the same effect on all varieties as described above for C. P. 28/60.

Strains 2 and 4 have been obtained from almost all sections of Louisiana, although there seems to be a tendency for one or the other to predominate in certain areas, while strain 1 has been identified chiefly from Canal Point seedlings at the United States Sugar Plant Field Station at Houma and in one isolated area about 200 miles away. The occurrence of strain 3 has been limited to Rosewood Plantation, where it was found originally, with the exception of a very few isolated, single stools.

Evidence of the probable existence of additional strains of the sugarcane mosaic virus in Louisiana is rather plentiful. One virus source, that had been identified as strain 2, was observed to produce a new type of necrosis on the older leaves of infected plants of the variety Co. 281. Another source, that had been called strain 4, was also used to inoculate Co. 281. Cuttings from this material exhibited about 40 per cent "germination recovery" which, previously, had been practically non-occurring in this variety. These two instances indicate definite differences between each of these virus sources and the four described above and given numerical designations. Still a third possibility of a different strain is the sudden appearance, in a single cane field, of quite an appreciable mosaic infection on C. P. 807, a variety long considered immune to the disease. Identification of this source of the virus is, at present, held up by failure to obtain infection when the usual technique is employed. Similar difficulties have been encountered with juice from other resistant varieties and the discovery of a procedure for securing juice, comparable in infectiousness with that from susceptible varieties, from such varieties would be of great value.

Careful observations of symptom patterns on many varieties, in the field as well as in greenhouse inoculations, are being made with the pur-

¹ Original thesis submitted August, 1935. Doctoral thesis number 339.

pose in mind of discovering new differential host varieties that may aid in further resolving the present strains or, more likely, in differentiating other virus sources that are already suspected or known to be different from the present strains. The thousands of seedlings now available probably offer the greatest promise as new differential hosts because of the extreme heterozygosity represented in their respective genetic make-ups. Fortunately, since sugarcane is propagated only by cuttings, each clone can be preserved and any peculiar utility it might offer as a differential host, due to its heterozygous conditions, can be made permanently available.

The three P. O. J. varieties, 36-M, 213 and 234, were 100 per cent mosaic in most Louisiana cane fields in 1925. By 1930, the disease had almost entirely disappeared from all plantings of P. O. J. 213, and was materially lessened in the other two, particularly 36-M, by what may be called the "recovery process," which consists of a combination of both "foliage" and "germination recovery." The latter is probably of much greater importance although the former has been definitely shown to be operative at times. A wave of secondary spread, beginning in 1930 and gaining in intensity during the succeeding years, has again brought infection to approximately 100 per cent in these varieties. During the early part of this period both types of recovery were common in P. O. J. 36-M, the average germination recovery with pedigreed-mosaic cuttings was over 50 per cent, and occurred to lesser extents in P. O. J. 234. Certain lines of P. O. J. 36-M showed significantly more recovery than other pedigreed-mosaic lines. No recovery of either type, however, occurred with the newly-infected material of P. O. J. 213.

During this period before the demonstration of strains of the virus it seemed that the differential rates of recovery observed in P. O. J. 36-M could best be explained by the assumption of a qualitative attenuation of the virus and that P. O. J. 213 had been infected with a virulent source directly from wild grasses, to which had been ascribed the power to "step up" the virulence of the disease. Attenuation could not, however, be experimentally demonstrated and so the discovery of "strains" of the virus offered a new and apparently more tenable theory to explain this phenomenon. In other words, a variety infected with one strain may be able to throw it off and recover but if infected with a different strain would be able to produce only diseased offspring. The virus that infected P. O. J. 213, for instance, prior to 1925 was a different and, for this variety at least, a less tenacious strain than the one that infected this variety in 1930 and subsequently. Experiments are planned that will give definite information on this phase of the problem.

Sugarcane mosaic was probably introduced in the United States prior to 1913, but, until 1932, there had never been observed on any variety any greater variation in mosaic symptoms than could be ascribed to normal variation. There had, of course, been wide differences in symptom patterns on different varieties. Prior to 1913, when quarantine laws went into effect, promiscuous shipments of sugarcane cuttings had been received from all over the world and the same was apparently true of other sugarcane-growing countries. It seems unlikely, however, that the strains here treated were brought in from different countries and have maintained their identity ever since without detection. This is particu-

larly true of strain 3, which causes such severe symptoms on all varieties that it certainly could not have been present for so long without attracting some attention. It would also have been more widely distributed than it seems to be at the present time. A systematic survey and strain determination of the sugarcane mosaic virus in other countries that produce sugarcane, such as is now being conducted in Louisiana, would be invaluable in determining the possible source of individual strains as well as their world distribution. In view of the evidence as it appears now, it would seem that strains of the virus are arising locally, possibly within the cane plant or by passage through other gramineous hosts. These less-favored hosts, or the insect vector (*Aphis maidis* Fitch) or even some new vector, may be fractionating a compound virus that has hitherto existed as a mixture. This would not be without precedent among virus diseases of other plants. Mutations of a single original virus, assuming it to be a living entity, offers a further possible explanation.

STUDIES ON INSECT HEMOLYMPH WITH SPECIAL REFERENCE TO SOME FACTORS INFLUENCING MITOTICALLY DIVIDING CELLS¹

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Many conditions and substances have been reported to influence, favorably or unfavorably, multiplication of protozoa, of bacteria, of yeasts, of vertebrate and invertebrate embryonic cells, of normal and pathological cells in plants and animals, and of isolated tissue culture cells; but only four references to stimulation of mitosis of insect hemolymph cells have been found. In 1924 Paillot³ injected *Euproctis chrysorrhoea* larvae with emulsions of *Bacillus melolanthae non liquefaciens* and found that dividing amebocytes rose from three or four per thousand cells to thirty or forty. In 1925 and 1927 Iwasaki^{4, 5} used *Galleria mellonella* larvae in a study of the effects of temperature changes and injections of bacteria, peptone, albumin, and various vaseline emulsions on the mitotic counts. From a normal of one or two karyokinetic cells per thousand, counts as high as 136 per thousand were obtained with bacteria injections. He found also that cells other than hemolymph cells were not involved in the mitotic reaction. In 1933 Paillot⁶ analyzed his data from many insects and discovered that cells which he called macronucleocytes were the type which responded to stimuli. He believes the "caryocinétose" reaction is connected in some way with the secretion of antagonistic immunity principles by the phagocytic macronucleocytes.

In the research referred to below, the roach, *Blatta orientalis*, was used as an experimental insect in a study of its hemolymph cell response to various conditions, to injections of a number of substances (enumerated later), and to inoculations with several different bacteria. Large nymphs or adults, male or female, were selected randomly from stock colonies, kept under close observation for ten to fourteen days, and then used either as control or experimental animals.

Injections were made with a small syringe whose hollow needle was inserted through the coxa-femur conjunctival fold of a metathoracic leg. About one-twentieth cc. (approximately 10 per cent of the average body weight) was injected. Bacteria were introduced by inserting a dissecting needle, wet with the culture, into the body cavity through the conjunctival folds between the tergal plates of the abdomen, about midway between the mid-dorsal line and the side of the animal.

¹ Original thesis submitted December, 1935. Doctoral thesis number 349.

² Aided by grants from the Rockefeller Fluid Research Fund, administered through Iowa State College.

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⁶ Paillot, L'infection chez les insectes. 535 pp. Patissier, Paris (1933).

Hemolymph cell counts were made by the method described by Yeager and Tauber⁷, except that 2,000 cells were counted for each determination.

RESULTS

Approximately 250 insects were used. A total of 2,595 mitotically dividing cell (M.D.C.) counts, each of 2,000 cells, was made; consequently, summarizing percentages were based on a total of 5,190,000 counted cells. Counts of 2,000 cells were found by experiment to be the minimum number that could be used to give satisfactory and consistent results. Duplicate counts of 2,000 cells each on the same sample, or from different samples from the same animal, give data that agree within reasonable limits.

TABLE 1. Summary of results from control hemolymph cell counts

Description of animals	No. of insects	No. counts made	Range Pctg. M.D.C.	Average Pctg. M.D.C.
♂ Nymph control	4	96	0.00-0.45	0.203
♂ Adult control	3	67	0.05-0.40	0.186
♀ Nymph control	4	145	0.00-0.50	0.194
♀ Adult control	3	68	0.05-0.40	0.126
Other controls, sex and age not checked	16	192	0.00-0.40	0.172
All controls	30	568	0.00-0.50	0.186
Initial count of all experimental animals	124	124	0.00-0.45	0.196

Ranges of percentages of dividing cells for some other groups follow (obtained at room temperature unless otherwise noted): Ovipositing females, 0.05-0.40; molting animals, 0.00-1.00; at 5° C., 0.00-0.20; at 37° C., 0.20-1.30; injected with *Bacillus subtilis* suspensions, 0.00-0.40; injected with *Serratia marcescens* suspension, 0.00-0.35; inoculated with *Staphylococcus aureus*, 0.00-2.80; fed *Staph. aureus*, 0.05-1.50; natural coccus infection, 0.20-2.35; natural rod infection, 0.15-3.10; paralyzed animals, 0.20-1.85; animals with abnormally vacuolated cells, 0.35-2.05. Ranges of percentages following the injection of additional materials were: Standard bacteriological nutrient broth, 0.10-1.95; 5 per cent beef extract, 0.00-2.10; 10 per cent peptone, 0.00-1.60; chick embryo extract, 0.00-2.70; defibrinated rat blood, 0.00-1.20; 10 per cent hemoglobin (horse), 0.05-3.80; 10 per cent egg albumin, 0.35-0.70; 5 per cent aspartic or glutamic acids, glycocoll, alanine, or tryptophane, 0.00-0.55; 5 per cent cysteine, 0.00-2.50; 10 per cent glutathione, 0.00-1.10; 40U insulin, 0.30-5.30; 10 per cent di-nitrophenol, 0.05-1.50; thyroxin (1 grain in 10 cc.), 0.05-1.90; 10 per cent glucose, 0.05-0.40; 1 per cent PbCl₂, 0.05-0.40; 1 per cent rotenone, 0.00-0.55.

Based on 5,190,000 cells, percentages of dividing cells in terms of mitotic phases are: prophase, 0.130; metaphase, 0.026; anaphase, 0.089;

⁷ Yeager and Tauber, Proc. Soc. Expt. Biol. and Med., 30: 861-863 (1933).

and telophase, 0.154. Percentage of amitotic cells was 0.0066; multinucleate (two to six nuclear fragments) cells, 0.026.

DISCUSSION AND CONCLUSIONS

Considering the large amount of data collected from normal control animals and from preliminary control periods for experimentals, it seems evident that a normal specimen of *Blatta orientalis*, be it male or female, adult or large nymph, has a M.D.C. count within the limits of 0.00-0.50 per cent. Counts of 0.00 per cent (less than one in 2,000 cells) were listed 43 times in the 604 control determinations made; 0.50 per cent only three times. Controls kept over 132 days maintained counts within the above range. The slight hemorrhages connected with the sampling of the hemolymph evidently did not influence the mitotic count. The range of average values (from 0.116 per cent to 0.250 per cent) might lead one to suspect some factor or factors of influencing various specimens. However, high and low averages were found among males, females, adults, and large nymphs, without relationship to the sex and age factors mentioned, or to some other factors which were controlled.

Although no connection between daily fluctuations in counts and daily changes in room temperature was found, a low temperature of 5° C. did decrease the mitotic evaluation, and subjection to a continuous temperature of 37° C. resulted in a decided increase in count values.

Since Tauber and Yeager⁸ had pointed out that high total hemolymph cell counts seemed associated with egg formation in crickets and other insects, a check was made to see if high M.D.C. counts were also an accompaniment of the condition. No deviation from the normal value was found, however. On the other hand, ecdysis, another physiological activity, is found to be associated with changes in dividing cell counts. Ecdysis is preceded by and accompanied by a distinct decrease in the percentage of mitotic cells. After molting the count tends to remain low for about a day, then increases, and usually reaches an optimum (as high as 1.0 per cent) on the third day. On the fourth day the count decreases and on the fifth or following days comes within the normal range. Reiche⁹ has shown that tissue autolysates favor mitosis; in ecdysis the cytolytic products from tissue breakdown may contain some stimulating substance.

With the exception of *Staphylococcus aureus* this species of roach seemed quite resistant to the bacteria injected or inoculated into its hemocoel. Susceptibility to *Staph. aureus* was very pronounced, and multiplication of the invading organism was accompanied by a rise in the M.D.C. values. The infection was also associated with definite external and internal symptoms, often terminated by death. The body became convexly arched and hemolymph cells became "ragged" and highly vacuolated. In connection with this phase of the study, an interesting observation was made. After death due to causes other than bacterial infection, hemolymph of control or experimental animals would, in many cases, become filled with bacteria within a short time, though none had been seen before the termination of activities. Apparently a latent or controlled infection

⁸ Tauber and Yeager, Iowa State College Jour. Sci., 9:13-24 (1934).

⁹ Reiche, Ztschr. f. Bot., 16:241-250 (1924).

had been present all the time and broke out as soon as the checking mechanism ceased to function.

Clues as to how natural bacterial infections may be spread were uncovered. Normal, uninfected animals given access to food soaked in cultures of *Staphylococcus aureus* often became diseased. Presumably the bacteria entered the hemolymph either through the gut wall, or through breaks in the exoskeleton. When one remembers the roach's cannibalistic habits, it is clear how diseases may pass from one animal to another.

Symptoms in those animals suffering with either of the two naturally occurring bacteria are much the same as indicated for *Staphylococcus aureus*. One difference was seen. In the last stages of the natural infections, conjunctival folds at various joints broke, and the thick, white hemolymph oozed out. The coccus and rod organisms can be transferred to normal animals by needle inoculations, and recovered from the hemocoelic fluid.

Increased M.D.C. figures obtained with injections of glutathione, cysteine, insulin, and other sulfur-containing compounds are interesting in view of Hammett's recent theory that sulfur as sulphydryl is the stimulating factor governing cell division¹⁰. Too, since glutathione is a constituent of muscle and blood¹¹, the rise in karyokinetic cell values following injections of blood, beef extract, peptone, and nutrient broth can also be explained by the same theory. Murray¹², likewise, has demonstrated a high glutathione content in chick embryos. Could results from injections of embryo extract be due to the presence of sulfur contained in glutathione? Hammett has published much data in support of his contention¹³, but, so far, has given no extensive explanation as to how the mechanism is stimulating to the mitokinetic process.

Dinitrophenol and thyroxin are drugs which cause increased metabolic activities of animals. The only clue that might explain their action on hemolymph cells may be related to the fact that the toxic products of increased katabolism in some way stimulate the karyokinetic activities.

In summary of the mitotic phases themselves it is seen that duration of the stages is in the following decreasing order: telophase, prophase, anaphase, and metaphase. The telophase is approximately six times as long as the metaphase.

Only 345, or 0.0066 per cent, amitotic cells were observed. Evidently this method of multiplication could be of little use in the maintaining of hemolymph cell populations. Several cases of nuclear division without subsequent cytoplasmic fission were seen. These were probably stages in the formation of polynuclear cells.

¹⁰ Hammett, *Protoplasma*, 7: 297-322 (1929).

¹¹ Hunter, *Jour. Biol. Chem.*, 72: 133-167 (1926).

¹² Murray, *Jour. Gen. Physiol.*, 9: 621-624 (1926).

¹³ Hammett, *Science*, 79: 457 (1934).

SOME FACTORS INFLUENCING THE GROWTH AND RESPIRATION OF RHIZOBIUM¹

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Allison, Hoover and Burk (2) and Allison and Hoover (1) reported that several different species of the rhizobia were unable to make any appreciable growth in a synthetic (sugar-mineral-nitrate) medium prepared from highly purified materials. The failure to grow in such a medium was attributed to the absence of a factor essential for the respiration and growth of the organisms. This factor was designated "co-enzyme R." The stimulative effects of a wide variety of substances upon the growth of *Rhizobium* was attributed to the introduction of appreciable quantities of this co-enzyme.

Repetition of much of the work of Allison and Hoover confirmed their results. Repeated attempts to culture various species of the root nodule bacteria through several consecutive transfers in a mineral salts-KNO₃-sucrose c.p. medium were unsuccessful. The addition of such materials as an alcoholic extract of commercial cane sugar, or various plant extracts to the medium induced appreciable increases in growth. A study of the influence of the reaction of the medium upon oxygen consumption and growth of the rhizobia showed that the production of changes in pH of the medium by such substances could in no case account for the stimulative effects noted. Further studies indicated that the results of Allison and Hoover were at least partially due to the medium employed rather than to the inherent characteristics of the organisms. It was found that *Rh. meliloti*, *Rh. trifolii*, *Rh. leguminosarum* and *Rh. japonicum* were able to maintain growth when continuously cultured in mineral salts-sucrose c.p. media with NH₄Cl or asparagin as sources of nitrogen, but were unable to make any appreciable growth with KNO₃ as the nitrogen source. The work of Allyn and Baldwin (3, 4) as well as the results of the present investigation indicate that KNO₃ poises bacterial media at a potential so high as to be unfavorable for the nodule bacteria. The addition of iron to these media composed of highly purified materials was found to bring about considerable increases in the growth of several species of *Rhizobium* as well as of such common soil bacteria as *Azotobacter vinelandii* and *Bacillus subtilis*. The optimum concentration of iron for the growth of *Rh. meliloti* and *Rh. trifolii* was found to be 10 parts per million parts of medium. Ferric chloride promoted greater growth than ferrous sulfate.

The organisms were unable to attain maximum growth rates in any of the media studied which were composed entirely of purified materials. Aqueous extracts of yeast or alfalfa employed as nitrogen sources led to the greatest growth and oxygen utilization of the rhizobia. The addition of such substances as alcoholic extracts of cane sugar or cane

¹ Original thesis submitted June, 1936. Doctoral thesis number 364.

molasses, clear filtrates of old *Az. vinelandii* cultures, aqueous extracts of soils, cysteine, or thioglycollic acid to cultures, of *Rh. trifolii* and *Rh. meliloti* in mineral salts-sucrose c.p. media with KNO_3 or NH_4Cl as the source of nitrogen greatly increased their activity. Asparagin and aspartic acid were unable to replace such substances but acted as very readily available sources of nitrogen. The nodule bacteria were able to attack the amino group of asparagin with greater ease than the amid group. The two carboxyl groups of these compounds seemed to increase the availability of the amino group and also seemed to exert an additional stimulative effect upon oxygen utilization and growth.

The mean respiratory quotient of five species of *Rhizobium* for 24 hours was highly significantly lower in glucose media containing yeast extract as a source of nitrogen than in similar media with either NaNO_3 or NH_4Cl as the nitrogen source. The mean quotient in the asparagin glucose medium was significantly lower than in the NaNO_3 medium. In media containing no sugar, yeast extract and asparagin brought about a similar lowering of the mean respiratory quotient of the several species of organisms.

All of the various materials which were able to stimulate the growth and oxygen utilization of the rhizobia when added to KNO_3 or NH_4Cl -sucrose c.p. media also brought about a decrease in the respiratory quotient of *Rh. trifolii* for the first few hours after inoculation. Yeast and alfalfa extracts lowered the respiratory quotient of the organisms over a longer period of time than did the other materials. Since the respiratory quotient is derived from the ratio

$$\frac{\text{CO}_2 \text{ produced}}{\text{O}_2 \text{ consumed}},$$

any lowering of this value would indicate a change in the physiological activities of the organisms. The increase in oxygen consumption compared to CO_2 production, thus, indicates that the substances studied acted as reducing agents with respect to the nodule bacteria.

The similarity in the response of the organisms to the various substances added when compared to the addition of small quantities of cysteine, and the general effect of all of the stimulative materials in lowering the respiratory quotients of the organisms seem to justify the conclusion that one of the important functions of materials used as accessory growth substances for the rhizobia is the provision of an initial hydrogen donator. Such hydrogen donators, presumably, furnish the organisms with a small amount of a readily available initial source of energy which enables them to make adjustments which seem to be necessary when organisms are inoculated into a new medium. Measurements of oxidation-reduction potentials indicated that most of the substances studied reduced the potential of KNO_3 sucrose c.p. media.

The results of the present investigation seem to justify the following conclusions:

1. Rhizobia can be continuously cultured in synthetic media containing highly purified sugars without the addition of any complex, unidentified co-enzymes or accessory growth factors.

2. The inability of previous investigators to obtain growth of the legume bacteria in a synthetic KNO_3 -sucrose c.p. medium may be accounted for in two ways: (1) the medium employed was deficient in iron, and (2) potassium nitrate, in the concentrations used, poised the medium at a potential unfavorable for the respiration and growth of the organisms.

3. One of the important functions of materials used as accessory growth substances for *Rhizobium* seems to be to provide an initial hydrogen donator. The role of a hydrogen donator for the nodule bacteria appears to be at least twofold; (1) it tends to lower the oxidation-reduction potential of the medium, and (2) it furnishes the organisms with a readily available initial source of energy which enables them to make the necessary adjustments for the establishment of favorable growth conditions.

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FURAN MERCURIALS AND DERIVED TYPES¹

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A. ORIENTATION IN THE FURAN NUCLEUS

In the investigation of the mercury compounds of furan it has been found that the most useful method for determining the position occupied by the mercury-containing group has been the replacement of this group by a halogen. But before this method can be of use, it is necessary to know the position occupied by the halogen in the derived compound. Important compounds for use as reference compounds in orientation studies are so-called 3,5-dibromo-2-furoic acid, 3-bromo-2-furoic acid, 3,5-dichloro-2-furoic, 4,5-dichloro-2-furoic acid and 3-chloro-2-furoic acid². The structures of these compounds as assigned by Hill and coworkers are wrong. The true structures of the compounds are, respectively: 4,5-dibromo-2-furoic acid, 4-bromo-2-furoic acid, 4,5-dichloro-2-furoic acid, 3,5-dichloro-2-furoic acid, and 4-chloro-2-furoic acid. These structures have been proved to be correct in the following manner: 4-Bromo-2-furoic acid (Hill's 3-bromo-) which may be derived by reduction of one halogen atom of 4,5-dibromo-2-furoic acid (Hill's 3,5-dibromo-) was heated with cuprous cyanide, potassium cyanide, and water in a sealed tube at about 200° C. for several hours³. The product was 2,4-furandicarboxylic acid. It was identified by the melting points and mixed melting points of the free acid and the dimethyl ester. Significant yields were obtained and the structure of the end product is known. 4,5-Dibromo-2-furoic acid was decarboxylated to form 2,3-dibromofuran (b.p. 160.5°-162.5° C., D_{26}^{25} 2.117, n_D^{25} 1.5430). The halogen atoms of this compound were replaced in the same manner as in the case of 4-bromo-2-furoic acid. The product was 2,3-furandicarboxylic acid, the structure of which is known. It was identified by the melting points and mixed melting points of the free acid and the dimethyl ester.

By the same general method the chlorine atom of 4-chloro-2-furoic acid (Hill's 3-chloro-) was replaced by a carboxyl group. This compound may be obtained by reduction of 4,5-dichloro-2-furoic acid. Although the yield of 2,4-furandicarboxylic acid (and its dimethyl ester) was small, the starting material was pure and the reaction is reliable. Much supporting evidence exists.

The structures of the various monohalogenocrotonolactones obtained from these compounds by Hill and Cornelison⁴ are probably wrong. All α -halogenocrotonolactones are probably β -halogenocrotonolactones and vice versa. Support for this theory is found in the fact that the melting

¹ Original thesis submitted March, 1936. Doctoral thesis number 361.

² Hill and Sanger, *Proc. Am. Acad. Arts Sci.*, **21**, 135 (1885); Hill and Jackson, *Proc. Am. Acad. Arts Sci.*, **24**, 320 (1889).

³ Rosenmund and Struck, *Ber.*, **52B**, 1749 (1919).

⁴ Hill and Cornelison, *Am. Chem. J.*, **16**, 188 (1894).

point of the so-called α -phenylamidocrotonolactones obtained by Hill and Cornelison by the action of aniline on a so-called α -halogenocrotonolactone is nearly that of the β -phenylamidocrotonolactone obtained from aniline and tetriconic acid by Wolff and Schimpff⁵. The general mechanisms for the transformation of the halogenated furan compounds into the halogenocrotonolactones, as postulated by Hill and Cornelison can be employed when the correct structures for the furan compounds and the probable structures for the crotonolactones are substituted for those used by these authors.

The half amide of 2,3-furandicarboxylic acid (m.p. 288°-293° C.) was obtained in small amount when 2,3-dibromofuran was treated with cuprous cyanide, potassium cyanide, and water, as has been described. Whether the amido group is in the 2-position or the 3-position is not definitely known.

3,5-Dichloro-2-furoic acid was reduced to 3-chloro-2-furoic acid (m.p. 153.5°-154.5° C.).

B. FURAN MERCURIALS AND DERIVED TYPES

In the chemistry of furan, compounds having mercury attached to the nucleus are of both theoretical and practical importance. The ease with which furan has been mercurated is a factor tending toward belief that furan has superaromatic properties². In a practical way mercurials have been found to be excellent derivatives for the identification and stabilization of some furan compounds.

Ethyl 2-methyl-4,5-dichloromercuri-3-furoate was prepared by refluxing ethyl 2-methyl-3-furoate with a buffered solution of mercuric chloride. By replacement of the chloromercuric groups with bromine, followed by hydrolysis, there was obtained 2-methyl-4,5-dibromo-3-furoic acid (m.p. 186°-189° C.). By oxidation of the methyl group 4,5-dibromo-2,3-furandicarboxylic acid (m.p. 242°-243° C.) was obtained. By substitution of one hydrogen of the methyl group by bromine, followed by hydrolysis, there was produced 2-hydroxymethyl-4,5-dibromo-3-furoic acid (m.p. 195°-198° C.); acetate (m.p. 143°-146° C.). By replacement of the α -bromine atom of 2,3-dibromofuran there is derived 2-nitro-3-bromofuran (m.p. 74.5°-76° C.), short, thick needles. By means of the general method of Gilman and Wright⁶ there were derived from the corresponding carboxylic acids 4,5-dichloro-2-chloromercurifuran (m.p. 182°-182.5° C.) and 3,5-dichloro-2-chloromercurifuran (m.p. 123°-124° C.). Methyl 5-bromo-2-furoate was mercurated by fusion with mercuric acetate and converted to the chloromercuri compound. The resulting methyl 4-chloromercuri-5-bromo-2-furoate (m.p. 234°-235° C.) was converted to known 4,5-dibromo-2-furoic acid by treatment with bromine followed by hydrolysis. No ketone was obtained when the mercurial was treated with ketene. Acetyl chloride under pressure and at elevated temperature split the mercurial and at the same time replaced the bromine atom with a chlorine atom. The final product was methyl 5-chloro-2-furoate (m.p. 40°-42° C.), which was further identified by hydrolysis to the acid.

⁵ Wolff and Schimpff, *Ann.*, **315**, 151 (1901).

⁶ Gilman and Wright, *J. Am. Chem. Soc.*, **55**, 3302 (1933).

The same result was achieved when methyl 5-bromo-2-furoate was heated in a sealed tube with mercuric chloride and acetyl chloride. Occasionally some hydrolysis to 5-chloro-2-furoic acid took place during the reaction. The reaction did not succeed with methyl *p*-bromobenzoate. When methyl 4,5-dibromo-2-furoate was treated with acetyl chloride and mercuric chloride in the same manner, what was probably 4-bromo-5-chloro-2-furoic acid (m.p. 150°-152° C.) was obtained upon hydrolysis. Methyl 4-iodo-5-bromo-2-furoate (m.p. 69°-69.5° C.) was obtained when the chloromercuri group of methyl 4-chloromercuri-5-bromo-2-furoate was replaced by iodine.

Methyl 4-chloromercuri-5-chloro-2-furoate (m.p. 215°-217° C.) was prepared from methyl 5-chloro-2-furoate in the same manner as was employed with the bromine analog. By treatment of this mercurial with bromine, followed by hydrolysis, what was probably 4-bromo-5-chloro-2-furoic acid (see above) was produced.

The results of mercuration of furan compounds indicate that mercuration proceeds in the β -position in accordance with the rules governing orientation in the benzene nucleus. In a furan compound having a *m*-orienting group in the 2-position and an *o*-, *p*-orienting group in the 5-position, the 4-position is apparently assumed by an entering mercury-containing group.

I. THE PRODUCTION OF PAPER FROM CEREAL STRAWS

II. THE UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION OF MISCELLANEOUS FABRICATED MATERIALS¹

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I. THE PRODUCTION OF PAPER FROM CEREAL STRAWS

There are in excess of 150,000,000 tons of cornstalks and 70,000,000 tons of cereal straws produced annually in the United States. These materials have an approximate general analysis of 35 to 45 per cent cellulose, resembling wood and cotton cellulose; 15 to 25 per cent pentosan, a source of xylose, furfural, and moulded plastics; and 25 to 35 per cent lignin, a material resembling protein in reaction. Laboratory and commercial trials demonstrated that a satisfactory cellulose for paper and rayon could be made from this source with less drastic and more economical processing than from wood.

This thesis covers the manufacture of paper from cereal straws and cornstalks by the kraft, soda, and other processes, using commercial paper manufacturing methods modified to the extent required by the physical character of these materials. The studies were conducted at the U. S. Bureau of Standards, and are included in the projects of the Agricultural By-Products Laboratory, U. S. Department of Agriculture, at Ames, Iowa. These government bureaus cooperated with the Iowa Engineering Experiment Station.

EXPERIMENTAL

At the conclusion of the first two years' work on paper, in which the digestion of wheat, oat, and rye straws by the kraft process was studied, a process was outlined and calculations made for the design of a commercial paper mill. Equipment for continuous digestion had the advantage of about 25 per cent reduction in digester capacity and increased thermal efficiency.

The next phase of the paper research was the production of paper from the cortex of the cornstalk. The first part duplicated the procedure on cereal straws in which the optimum range of conditions, for the dual digestion with water and chemical, were determined. A study was also made on the repeated use of single batches of water digestion liquor and chemical digestion liquor, to concentrate the extracted material for practical utilization or chemical recovery.

The second part of the cortex studies was the semi-commercial digestion of larger batches of pulp for paper machine runs at the Bureau of Standards in Washington, D. C. The digestions were made in a three-foot spherical rotary digester using direct steam, whereas the laboratory scale

¹ Original thesis submitted July, 1935. Doctoral thesis number 328.

studies were made with indirect steam. Difficulties were experienced with variable dilution from the direct steam and the variable quantity of bone-dry fiber. The dilution difficulties were overcome by a study of steam consumption and radiation. Difficulties with the determination of the quantity of bone-dry fiber were overcome by establishing an empirical relation between the measured cortex displacement of water in the digester and its bone-dry fiber content. It was found that the degree of digestion and refining, and the cleanliness of the pulp, could be judged from the examination of hand sheets under moderate microscope magnification by transmitted light.

The cortex fiber of cornstalks hydrates, or gelatinizes, abruptly in contrast to wood fiber. This characteristic makes cornstalks particularly adaptable to glassine papers. Laboratory scale studies on hydrating cortex from soda digestions were made.

CONCLUSIONS

1. Paper, varying from wrapping paper to a good grade of bond and glassine papers, can be made from cereal straws and cornstalk cortex, by a water digestion, modified kraft or soda cook, combined beating and bleaching, and cleaning by screening and centrifining.
2. The strength of the paper varies from strongest with oat straw, through barley and wheat, to the weakest with rye straw. Bleachability varies in the reverse order.
3. The calculations for a proposed paper mill indicate the advantage of the re-use of the digesting liquors and of a continuous digester.
4. In using a rotary digester, digestion can be controlled from steam consumption data and from a displacement measurement of the wet fiber.
5. The quality of the hand sheets was controlled by the microscope, using moderate magnification and transmitted light.

II UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION OF MISCELLANEOUS FABRICATED MATERIALS

In the course of experimental studies on agricultural wastes, by the U. S. Bureau of Standards and the Iowa Engineering Experiment Station at Ames, Iowa, the manufacture of diverse products from these materials was studied for practical outlets for substantial quantities of these wastes. Some of these products were new and required inventive faculty, and all the products required adaptation to the distinct characteristics of the agricultural wastes.

This thesis covers phases of the development of several such products.

EXPERIMENTAL

Cornstalks, which possess two types of fibers with widely different physical characteristics, had previously been separated into two fractions by both wet and dry processes. The separation by each method was studied and these separations were distinctly improved and adapted to better commercial processing. The best wet procedure was found to be shredding the stalks, scrubbing with four or five changes of water, drain-

ing, treatment in an attrition mill, floating the pith and fine fiber up through a screen by means of an upward flow of water, and a final continuous flotation of the pith. A dry attrition treatment was also developed which was followed by air separation, either over an inclined baffle or in a centrifugal air separator.

A process for pressing and moulding complicated shapes, such as window sashes and fiber pipes, as unit structures was developed. The material was waterproofed to a high degree, the products conditioned with moist heat to prevent warping, and tested to demonstrate their strength as unit structures.

A method of solvent sizing pressed fiber products by impregnating the dry fiber with a solution of paraffin or wax in carbon tetrachloride, gasoline, or other solvents, and evaporating the solvent, was developed. The degree of waterproofing was tested by both the standard and special deflection and immersion test.

A method was perfected of determining the moisture content of pressboard in the hydraulic press by measuring the electrical conductivity.

The conductometric method of determining end points in volumetric titrations was applied to the spent liquors from paper making, using practical equipment and simplified procedure. The test was also applied to the titration of pyroligneous acid and acid distillate from the destructive distillation of agricultural wastes.

The precipitation of organic matter from the spent digesting liquors from paper making was studied using acids and soluble salts of Ca, Br, and Sr. The organic material precipitated readily as a heavy filterable floc. The filtrate was clear, the color depending on the precipitating agent. This suggested a procedure whereby the precipitate could be pressed, dried, and the filtrate causticized for further digestions.

CONCLUSIONS

1. Cornstalk cortex and pith can be divided by wet, moist, or dry attrition treatment, and either water flotation or air separation, with commercial equipment. The cortex is satisfactory for cellulose uses. The pith has a higher thermal insulation value than cork.
2. Complicated forms of cornstalk fiber products can be pressed and moulded as unit structures. These can be cheaply made and excel similar wood products in strength.
3. Cornstalk pressboard is made very moisture resistant by solvent sizing. A deflection and immersion procedure was developed as a test for waterproofing.
4. The change in electrical conductivity can be used to determine the moisture in pressboard and this method is suited to research and technical control. It also shows defects in press design.
5. Conductometric titration can be applied to advantage in the analysis of spent liquor from pulp digestions.

THE ENDOGENOUS PHASES OF THE LIFE CYCLES OF EIMERIA NIESCHULZI, EIMERIA SEPARATA, AND EIMERIA MIYAIRII COCCIDIAN PARASITES OF THE RAT¹

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Experimental work in parasitology, as in many other fields of biological endeavor, can be done effectively and judiciously only after the complete life histories of the organisms involved have been discovered. Although the knowledge derived from studying the life cycle may assist but little in direct control of a parasite, the fundamental information thus derived is essential for intelligent experimentation on the best method of control. The rat is a laboratory animal that is well adapted in many ways for experimental work in coccidiosis. Furthermore, it harbors three species of coccidian parasites, whose endogenous cycles have never been delineated. An accurate comparative account of these cycles should constitute an important basic contribution to a program for the study of host-parasite relationships in coccidiosis.

For these reasons a study of the life cycles of the three coccidian parasites of the rat was undertaken.

HISTORICAL

Schaudinn (1900) was the first worker to elucidate the entire life history of a coccidium of the genus *Eimeria*. His work was an important step forward in the study of the coccidia, but was actually not complete in the light of what has since been uncovered concerning the histories of these parasites. Tyzzer (1929), and Tyzzer, Theiler and Jones (1932) have published most complete and understandable studies on the *Eimeria* of gallinaceous birds.

Dieben (1924) made some observations on the life cycle of a rat coccidium, *Eimeria nieschulzi*, which he was describing.

The contributions of Tyzzer, and Tyzzer, Theiler and Jones are stimulating and suggestive for the investigation of other species of *Eimeria*, but since their work was done on several species from the chicken it is felt that in the present instance there is no necessity to make close comparisons between the developmental cycles in the two hosts. The fact that different hosts are involved is sufficient to necessitate individualization of data.

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²The author wishes to thank Dr. E. R. Becker for his suggestion of the problem, for his continued encouragement, and for his advice and criticism during the entirety of the work. Without his guidance the solution of the problem would have been all but impossible.

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MATERIALS AND METHODS

All three of the known *Eimeria* of the rat were employed in this study. Each formed the basis for a distinct portion of the experimentation. No attempt was made to have all of the host animals from the same strain, since it was felt that the strain of rat would have little or no effect on the cycle of the parasite. It was known, however, that the rats had not been infected previous to the experimental infection. This precaution was necessary in order that no so-called "hold-over" infection could be present, and so that the animal would not be infected, experimentally, with less than the maximum number of parasites.

Infection, in most cases, was accomplished by injecting into the stomach, through a rubber catheter, a known number of sporulated oöcysts. The number of oöcysts given each rat varied with the length of time the host was required to live. For example, a much larger dose was given to animals intended to be killed on the first day of infection than those intended to be used for study on subsequent days. This gradation in infective dose was made to insure, first, that the animal survived until the correct time, and second, that the infection was as heavy as possible in order to facilitate the study.

The oöcysts used in the present work were derived from various sources. The oöcysts of *Eimeria nieschulzi* were the progeny of the strain inbred by Hall (1934); oöcysts of *Eimeria miyairii* were derived from the culture sent to Dr. E. R. Becker by Dr. A. C. Chandler; the *Eimeria separata* oöcysts were collected by the author.

Material for study was secured from rats at 24-hour intervals and when necessary at intervals less than 24 hours. From each rat, five pieces of intestine were collected. These portions were taken from the upper small intestine, middle small intestine, lower small intestine, caecum and colon. The tissues were fixed in Zenker's, dehydrated, sectioned and stained with Goldhorn's polychrome methylene blue and eosin. Other stains were used, but, for the most of the work, it was found that the stain mentioned was the most useful.

At the same time that the tissues were fixed, living material was studied from the various levels of the intestine and smears were made from those portions of the intestine which showed parasites. In the case of early infections, smears were made not at levels where parasites were found, but at levels where experience showed the infection could be expected. This was done because some of the earliest stages in the life history were not observed in living condition. The smears obtained were fixed in Schaudinn's and stained with iron haematoxylin and, in some cases, with Goldhorn's polychrome methylene blue and eosin.

Study of the microgametes were made using smears similar to those just described and also with two other types of preparations. The first of these were made by placing a drop of blood from the host on a clean slide and hurriedly smearing it with a piece of the intestine in which living gametes had been found. The slides were then dried and stained with Wright's blood stain in the usual manner. These preparations served to show the flagella to a distinct advantage. The second method consisted of mixing a little of the intestinal contents on a slide in a drop of two per cent aqueous Congo red. This drop was spread on the slide and allowed to dry. When thoroughly dry it was placed in 95 per cent ethyl alcohol to which

several drops of hydrochloric acid had been added. This procedure stains the background black, while the flagella and body stand out as white objects on the black background.

Since the greatest difficulty presented by the study of the life cycles was the finding and identifying of various generations of merozoites, it may be well to explain the method employed.

Stained smears were studied and each type of merozoite found was drawn with the aid of a camera lucida. This study was made for each interval of time and for every rat infected. The resultant figures were all laid side by side and each merozoite from each rat matched with similar merozoites from other rats; for example, if four distinct types of merozoites were found on the fourth day, three on the third, and two on the second, the similar merozoites found on the fourth, third and second days were all placed together, thus indicating a single type or generation. By this process of elimination the first type of merozoite to appear was discovered and designated the first generation, the second type derived was called the second generation, until all possible types had been found. At the same time it was noted on what day each new type was first discovered, and thus it was found when each new generation appeared in the lumen of the intestine. The data accumulated were checked with the sections of the intestine. Specimens of each generation, on smears, were then drawn and measured to obtain the mean and range in size.

The number of merozoites in each group was obtained by counting the number of nuclei present only after the merozoites had been fully formed. This was useful especially when more than one section had to be examined to get the complete number in one group, because it prevented duplication in count as it was not likely that the same nucleus could occur on more than one section.

NOMENCLATURE

The first authentic description of a coccidium from the rat, *Eimeria miyairii*, was by Ohira (1912). Since this article is not available in English, previous workers were guided in determining species entirely by Fukuhara's brief German abstract, and by the fact that Pérard (1926) accepted the above name for the parasite with which he worked. Recently the author obtained a photostatic copy of parts of the original paper by Ohira and, by comparing the figures with the material used in the present study, found that the coccidium previously considered to be *E. miyairii* was not that described by Ohira. A comparison of the macrogametocytes of the parasite described by Ohira with the macrogametocytes of Pinto's (1928) *E. carinii* reveals that they are the same. Therefore, the coccidium discussed by Becker (1934) as *E. carinii* is actually *E. miyairii*. This correction leaves for the parasite considered by Becker (1934) to be *E. miyairii* the name *E. nieschulzi* Dieben (1924), the correct appellation according to the rules of nomenclature.

It should be mentioned that Becker and others in several papers (1932, 1934) noted the possibility that the names used were the incorrect ones.

The correction made does not change the status of *Eimeria separata* Becker and Hall (1931), which remains valid as described.

Eimeria halli Yakimoff (1935) remains an uncertain species since the measurements are conflicting with the species mentioned in the foregoing paragraphs. The method used in procuring the material and the lack of experimental data, also, should cause some doubt as to its validity.

EXPERIMENTAL

ENDOGENOUS CYCLE OF *EIMERIA NIESCHULZI*

Eimeria nieschulzi Dieben (1924) is a parasite of the epithelium of the small intestine of the rat. The infections of this species are heaviest in the middle of the small intestine. The epithelial cells most affected are found along the base of the villi and in the glands, but usually not as far as the fundus of the gland. In several cases, however, schizonts were found at the base of the glands and even in the Paneth cells. The position of the schizonts is generally toward the distal end of the cell, but variation has been found in all of the generations of schizonts. The macrogametocytes are found, in the vast majority of cases, toward the proximal end of the parasitized cell.

Dieben, in the description of *E. nieschulzi*, reports the prepatent period to be eight days, while Becker and Hall (1931) find it to be seven days. The latter is in accordance with the author's findings.

Dieben states that the parasite is found in the small intestine and also in the caecum. Since the present worker has been unable to find phases of the life history of this parasite in the caecum it is supposed that Dieben was working with a mixed culture of both *E. nieschulzi* and *E. separata*, the latter being the form found in the caecum. However, since the majority of forms described by Dieben compare with those found to be parts of the cycle of *E. nieschulzi*, we are considering the form from the small intestine to be the parasite described by that author.

Becker (1934) gives the location of the coccidium under consideration as being in the small intestine, caecum and colon, and gives the present author credit for the information. The information given was intended only for the other two species in the rat.

Since the purpose of this work was to study the endogenous phases of the life cycle, the description will necessarily begin with the sporozoite after excystation in the intestine. Time, when mentioned, is the shortest period obtained, since the minimum time is the time desired in this type of life history survey.

From three to four hours after infecting a rat with *E. nieschulzi* the sporozoites may be found in the lumen of the small intestine. These sporozoites may remain in the intestine in infective condition as long as four days. This statement may be substantiated by the fact that smears, made four days after a single infection, showed sporozoites in the lumen of the small intestine. Moreover, tissue from this rat showed very young first generation schizonts in some of the cells. Thus, it is shown that a single infection *per os* actually means a continued infection for four days when the actual entrance of the sporozoite into a cell is considered. The continuous infection for four days accounts for the fact that the patent period is generally four days in length. Hall (1934) found, however, that in approximately 50 per cent of the infections carried on by her the patent period was five days in duration. Since the patent period is calculated from the appearance and disappearance of oöcysts in the feces, it must

be understood that it takes some time for the oöcysts to be eliminated from the body. This time would be a factor in the apparent lengthening of the patent period. Further, sporozoites may, possibly, live in some rats longer than four days, since they were present, at that time, in numbers great enough to allow them to be found on smears. A carmine suspension fed to rats with a stomach tube was not totally eliminated until slightly less than four days after feeding. (Pellets, taken at six-hour intervals, and mashed in 70 per cent alcohol gave a red tinge to the alcohol until almost the fourth day.) It is believed that motile sporozoites could remain in the intestine slightly longer than inert particles of carmine.

Hall (1934) suggests that the increased length of oöcyst elimination with increase of the size of the infective dose might be because the greater number of oöcysts being passed allowed them to be found later than usually. This may be a factor, but it is not the entire explanation. In the light of the above discussion it is believed that the greater the number of sporozoites present the greater the chance that some of them might not contact a suitable host cell until a relatively late time, thus the patent period would be lengthened. Subsequent generations of merozoites also may have difficulty in reaching host cells, and thus lengthen the cycle.

The continued infection caused by the entrance of sporozoites over a period of time necessarily causes some complication in the life history. It has been shown that the cycle is beginning and ending over a period of approximately four days. Also, each generation of merozoites may be expected to be found on a similar period of four days. This shows that on any single day several generations of merozoites may be present. The method of separating and identifying these generations has already been discussed.

Because the prepatent period is seven days, it should be noted that the oöcysts must leave the tissues at approximately six and one-half days in order to get to the outside on the seventh day. Therefore, the time for minimum endogenous development is six and one-half days, but the prepatent period ends at the time the oöcysts appear in the feces. Thus it is shown that the endogenous development does not end with the prepatent period. However, since it is the latter from which calculations have been made by other workers, it is considered that the seventh day, or the time when the oöcysts first appear in the feces, is the termination of the cycle. For the period actually spent in the tissues, the name endogenous period is suggested in order to distinguish it from the prepatent period.

Sporozoites of *E. nieschulzi* when excysted range in length from 9.9-12.15 μ , the mean length being 11.33 μ . Width, at the nucleus, varies from 1.15-2.25 μ , with a mean of 1.8 μ . When stained with iron haematoxylin the nucleus shows a well-defined central karyosome with a very light staining nuclear wall. The sporozoite contains two globules which are extremely siderophilic; the anterior one being circular in outline, the posterior being elongate oval. These globules, when stained with Goldhorn's polychrome methylene blue and eosin, take an intense red color. One sporozoite was found in which the posterior globule was divided in two, each part being circular in outline. This particular specimen had a rather peculiar nuclear structure, the chromatin being divided in three pieces, giving them the appearance of chromosomes. Since no other similar specimen was found it is considered that it was not a normal condition. (Pl. I, Figs. 1-2.)

The sporozoite, after entering the host cell, forms the first generation schizont. This schizont may be distinguished from later generations by the presence of a large refractile globule. The two globules of the sporozoite evidently unite after the sporozoite has entered the cell, thus giving rise to the characteristic globule. Like the two from which it is derived, the globule of the schizont stains intensely red with eosin. The eosinophilic mass may be seen almost up to the time the merozoites are completely formed, but at some time before actual maturity of the merozoites it disappears. As long as the globule can be seen it is surrounded by the residual mass of the schizont. The first schizogony is complete at about 36 hours, and from the first schizont 20-36 merozoites are formed. (Pl. I, Figs. 3-4.)

The merozoites formed are known as the first generation merozoites and have a range in length from $6.75\text{--}9.9\mu$, mean length being 8.59μ . They vary in width from $1.35\text{--}1.98\mu$, mean being 1.64μ . When seen on smears stained with iron haematoxylin these merozoites have a granular cytoplasm; the majority of them show a rather deeply stained anterior end. Tissues stained with polychrome methylene blue and eosin show these merozoites with two small eosinophilic globules—one anterior and one posterior to the nucleus. These globules are so strikingly similar to the globule of the sporozoite that one is led to think that they are made of the same material—perhaps these are derived from those of the sporozoite. Study, however, fails to find any trace of them on the smears stained with iron haematoxylin, so it is believed that they must be derived from some other source. (Pl. I, Figs. 5-7.)

First generation merozoites, after breaking out of the host cell, migrate to and enter other intestinal cells. Here form the second generation schizonts. This schizont is characterized by having the red globules of the first generation merozoite imbedded in its cytoplasm. Both globules may sometimes be seen; at other times, only one; and some instances have been noted where neither is to be seen. The latter case may be because of the manner in which the section had been cut. The second schizont matures in about 48 hours after infection and gives rise to 10-14 second generation merozoites, which have a length of $12.6\text{--}16.2\mu$, mean 14.36μ , and a width varying from $0.9\text{--}1.4\mu$, average being 1.24μ . With iron haematoxylin they show a granular cytoplasm which stains relatively lightly in the region around the nucleus. Some groups of merozoites, evidently belonging to this same generation, when seen in sectioned material, showed an eosinophilic mass near the posterior end. Only a few of these, however, have been seen. (Pl. I, Figs. 8-11.)

Third generation schizonts are initiated when the second generation merozoites enter the epithelial cells. So far as can be determined no distinguishing morphological feature is present in this schizont which would differentiate it from the early fourth generation. The time of its appearance may assist in identifying it only in material in which it is certain the fourth generation has not as yet begun. The difference in the number of nuclei present in the older fourth generation schizont is a feature which is not of much assistance. Third generation schizonts give rise to 8-20 merozoites which break out of the cells on the third day. These merozoites range in length from $17.1\text{--}21.6\mu$, the mean length being 19.08μ ; in width from $1.0\text{--}1.35\mu$, with a mean of 1.19μ . These merozoites are generally the first noted in living material and on smears because of their size.

They are characteristically bent and usually are seen shaped like a U or a J; this, however, is not true of all specimens as some are straight. In the anterior end of each merozoite of the third generation there is a densely granular portion. There are also, in the anterior portion, along the median line, several larger granules—usually three in number. These features, along with size, assist in separating the third from the second generation. (Pl. II, Figs. 1-2.)

Fourth generation schizonts, formed by third generation merozoites, possess, in their later development, more nuclei than any other generation. The fourth generation schizont gives rise to merozoites on the fourth day of the infection. These merozoites, from 36-60 in number, have a length range of 4.5-6.7 μ , average being 5.5 μ . Width varies from 1.0-1.8 μ , with a mean of 1.35 μ . Size alone is a characterizing feature of this generation of merozoites. It should be mentioned that in the author's material these schizonts appeared on sections rather infrequently. Discussion of this point will be deferred until later. (Pl. II, Figs. 3-6.)

TABLE 1. *Eimeria nieschulzi*. Data concerning the asexual phases

	Sporozoite	Merozoites			
		1st generation	2nd generation	3rd generation	4th generation
Range	9.9-12.15 μ	6.75-9.9 μ	12.6-16.2 μ	17.1-21.6 μ	4.5-6.7 μ
Length Mean	11.33 μ	8.59 μ	14.36 μ	19.08 μ	5.5 μ
Range	1.15-2.25 μ	1.35-1.98 μ	0.9- 1.4 μ	1.0-1.35 μ	1.0- 1.8 μ
Width Mean	1.80 μ	1.64 μ	1.24 μ	1.19 μ	1.35 μ
Range		20-36	10-14	8-20	36-60
Number in one schizont Mean		26	12	15	50
Time of ma- turing		1.5 days	2 days	3 days	4 days

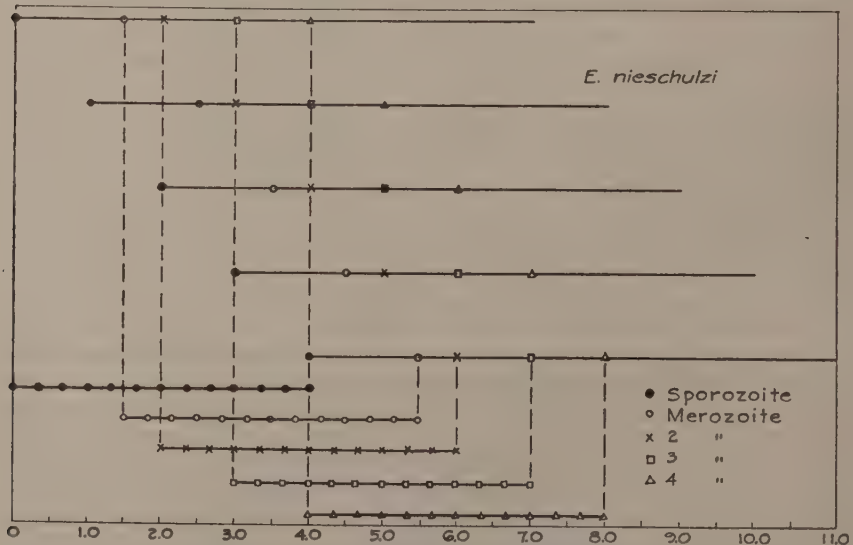
Gamete formation begins when the fourth generation merozoites enter host cells. The young gametocytes may first be noticed at about five and one-half days but cannot be identified until some time later because the distinction depends upon the division of the nucleus of the microgametocyte. Each young gametocyte consists of a central mass of chromatin around which appears a crescentic area which seems to be filled with a fluid—at least granules are not found in that area. Surrounding this area is a region of rather densely granular cytoplasm which extends to the cell wall.

The nucleus of the microgametocyte undergoes a number of divisions, during which time the cytoplasm increases in volume. These nuclei, which are formed from the division, later migrate to the periphery of the gametocyte. During and after the migration to the surface the chromatin masses which have, up to this time, been circular in outline, begin to elongate and become roughly triangular in shape. This shape changes, and when the gametes are mature they have a general outline similar to

that of a comma. When found on smears of intestinal contents their shapes vary considerably, depending upon the position they assumed at the time of fixation. The preparations described under the section on materials and methods, along with some observations on living material, serve nicely to show flagella. The measurements were made from specimens on smears stained with Wright's. This fact is important because the size is probably influenced by the fact that they were allowed to dry.

The microgametes typically have two flagella which arise at the anterior end of the body. At the base of the flagella in a few specimens stained with iron haematoxylin a small, round, dark body was observed. This object, seen also by Dieben (1924), the author considers to be a blepheroplast. The remainder of the body of the gamete appeared in the majority of cases to be, for the most part, homogeneous. The body proper of the microgametes had a mean length of 4.38μ and a width of 0.61μ ; the flagella averaged 9.18μ in length. (Pl. II, Figs. 7-10.)

The macrogametocytes of *Eimeria nieschulzi* occur in the same locality in the intestine as the asexual phases of the cycle. However, the macrogametocytes are usually found in the proximal end of the epithelial cells which they infect. The parasitized cells in infections with this species have never been found pushed out of the epithelial layer except when they contain the mature oöcyst, and double infections with macrogametocytes have never been encountered. These are specific characters. (Pl. II, Figs. 11-12.)



EXPLANATION OF LIFE HISTORY GRAPHS

The numbers on the abscissa indicate the day of the infection. Each solid line beginning at zero, one, two, etc., days represents the length of the prepatent period for the progeny of each sporozoite. Since sporozoites may infect on any of the first four days, the lines are found beginning up to and including the fourth day. Infection, however, begins at any time during that period, therefore the lines projected below more correctly illustrate the continuity of the infection. These projected lines show graphically when each phase of the life cycle may be found and at the same time show exactly what forms may be found in the intestine at any day of the infection.

It was not deemed essential to try to measure the macrogametocytes because age would cause so much variation in size and because they can be measured more nearly correct after they leave the animal as oöcysts.

The oöcyst wall begins to form early in the development of the macrogametocyte and since the construction of the wall is interesting, it is outlined in the following paragraph. The first change to take place in the development of the oöcyst wall is the formation of some granules in a circle around the nucleus. The first granules to develop are comparatively large and stain a dull red with eosin. The second are much smaller and stain an intense blue with methylene blue. The former granules are termed plastic granules; and the latter, haematoxylinophilic granules after Hosoda (1928). As the macrogametocyte grows older the plastic granules move toward the periphery of the cell and become flattened in a rather thick layer at the limits of the gametocyte. This layer forms the outer wall of the oöcyst. The haematoxylinophilic granules follow the plastic granules in their migration toward the limits of the cell, but do not reach the edge until the outer wall has been completely formed. Upon reaching the outer wall, these haematoxylinophilic granules begin to flatten out just as the plastic granules did. Since the former are smaller, the layer formed is much thinner. This layer derived from the haematoxylinophilic granules stains blue with methylene blue and eosin. (Pl. III, Figs. 1-6.)

During the time the oöcyst wall has been forming another group of granules similar to the plastic granules arises around the nucleus. These begin to migrate toward the periphery, but do not reach it; instead, they remain dispersed in the cytoplasm. It is not at all unlikely that this latter set of granules, dispersed throughout the entire cyst, gives to the unsporulated oöcyst its vacuolate appearance. It is probable also that these granules may have some part in the subsequent formation of the sporocyst wall, since this wall is usually made in one layer and resembles the outside wall of the oöcyst.

In order to be certain that the oöcyst wall is composed of only two layers some oöcysts were placed on a slide and crushed with the coverglass. Many of the oöcysts crushed had only the outside wall broken, thus showing a distinct thick outer wall and a thin inner wall or membrane.

Fertilization spindles have not been seen, but microgametes have been found in sections in close proximity to the wall of the macrogametocyte. Whether or not fertilization instigates oöcyst wall formation will be taken up subsequently.

When the oöcysts of *E. nieschulzi* are eliminated the host cell wall seems to break and the parasite is forced out. Only on rare occasions may mature oöcysts be found with a remnant of the host cell attached to it.

Henry (1932) pointed out that the oöcyst wall of *Eimeria* was usually composed of two, and less frequently three layers. She also noted that in the two layered walls the outer one was the thicker of the two. This is the same condition as noted above for the species under consideration. The figures prepared by most authors represent the wall of oöcysts by a thin outer line and a thick inner one. The oöcysts appear as described but an explanation should be made for such appearance. The thin outer line is actually the interface between the outside medium and the first layer

of the wall. The light area immediately inside of it must be interpreted to be the outer wall itself. Next, the heavy inner line must be the interface between the inner and outer layers; thus the dark portions in most figures represent the interfaces and the light refracted from them and not the layers of the wall itself.

From the foregoing outline of the life history of the coccidium under consideration it would seem plausible to conclude that at least this species of the genus *Eimeria* has a self-limited life cycle. There is, however, a possibility that every host reacts exactly the same to infection, and so it would seem that the host actually limited the cycle in one way or another. This has already been proven incorrect by the transmission of merozoites as carried on by the author (Roudabush, 1935). In the work mentioned the author infected a rat for five days and then transmitted the infection to a second rat, by means of the merozoites. If the cycle is host limited the second rat should have thrown off oöcysts on the seventh day after transmission; instead, the rat eliminated oöcysts on the second day after transmission, thus showing that the cycle is limited not by the host but by the parasite itself.

It has been pointed out that the fourth generation merozoites were not to be found in any great numbers and because of this fact two possibilities should be brought to mind. The first of these is the most likely and most obvious. The scarcity of the last generation merozoites may be only an apparent reduction in numbers because the material collected was not taken at the time of greatest production of these asexual phases. The other possibility is, that instead of having a fourth generation of merozoites this small sized group is a sexually differentiated portion of the third generation. Should this be true the larger merozoites, which have been called third generation, would be the ones giving rise to the macrogametocytes while the smaller merozoites being fewer in number would give rise to the male elements. This, of course, is difficult to prove because if a merozoite is found in a cell it may be correctly placed in the group of merozoites to which it belongs, but the product it is going to form cannot be assured. Conversely, when the product can be identified the merozoite from which it is derived cannot.

Before an accurate opinion is formed about each of the above possibilities consideration should be given to one other matter. This concerns itself with the number of oöcysts eliminated from a single infection of a known quantity. Hall (1934) in studies on the quantitative infections of this same species of parasite found that out of thirty-three attempts at single oöcyst infections only twenty-two were successful. From these data she concluded that two-thirds of the total number of oöcysts fed constituted the infective dose. This calculation, while probably mathematically correct, would seem to be biologically unsound. In order to be correct concerning the number of viable oöcysts in her cultures it would have been necessary to make all of the thirty-three infections on the same day from the same culture. Even then the possibility of the oöcyst remaining in the dilution pipette, or some other similar accident in technique, could not be guarded against sufficiently to be certain that every rat actually swallowed the oöcyst intended for it. Disregarding this latter statement and supposing that all of her attempts at single oöcyst infections were made from the same culture on the same day, to be correct about the remainder of the infections they would necessarily have had

to be made at the same time from the same culture. This, of course, would be improbable. In order to attempt a correction of the data compiled by Hall and to correlate it with the present work it is necessary to consider that every sporulated oöcyst fed is, disregarding the fact that cultures vary in viability, a potential infector. The author realizes that cultures vary in infectability because of age and environment; but since it is practically impossible to standardize each culture, if cultures are used when fresh, each sporulated oöcyst does constitute at least a potential infector.

In view of the preceding statements a recalculation of the means for yields from various infective doses is given here. These calculations are on the basis that six oöcysts fed is a six oöcyst infection.

	Yield per oöcyst fed
1 oöcyst fed	62,000
6 " "	1,455,000
15 " "	1,389,000
75 " "	1,098,000
150 " "	1,029,666
2000 " "	144,150

From these data it will be seen that the highest mean yielded from a single oöcyst is 1,455,000. This number, of course, represents the number of macrogametocytes formed during the course of the infection.

Multiplying the mean number of merozoites found in schizonts one, two, three and four and then multiplying that number by eight, the number of sporozoites in one oöcyst, it is found that each oöcyst fed produces a mean total of 1,872,000 gametocytes. Subtracting 1,455,000—the number of macrogametes as found in Hall's revised data, we have a total of 417,000 microgametocytes formed from one oöcyst. This number is slightly over 20 per cent of the total number of gametocytes formed, which is about the correct ratio as observed in sections of the intestine. The extremes of infection may be calculated by multiplying the minimum number of merozoites in each generation by each other and that result by eight, the sporozoite number, and by multiplying the maximum number in the same way. This calculation gives a range of 460,800-4,819,200 gametocytes produced from one oöcyst.

If the calculation made from Hall's and the author's data is correct, it is plausible to consider that the small sized merozoites belong to a distinct generation, since the average number in a single fourth generation schizont was used in the calculation. The author, for the present at least, considers that there are four complete generations of merozoites—but points out the other possibility, merely because it is a possibility.

Hosoda (1928) indicates in his work that fertilization takes place before the oöcyst wall has actually begun to form. Since fertilization has not been seen in the present work it is impossible to verify Hosoda's observation. It has been observed, however, that the formation of the granules which ultimately constitute the oöcyst wall takes place rather early. In addition to this, all of the oöcysts usually are eliminated and no hold-over macrogametocytes have been found. This would seem to indicate that either there is a one hundred per cent fertilization or else that all macrogametes after a time are thrown off by the host. It would seem

rather improbable that in every infection there would be one hundred per cent fertilization so that the latter of the two suppositions would be the more plausible. This again allows several possibilities, the first of which would be that the unfertilized macrogametes are in some manner destroyed by the host. Another possibility is that the oöcyst wall is formed without regard to fertilization and so some unfertilized oöcysts are eliminated by the host. The latter may account for the number of oöcysts which usually fail to sporulate.

ENDOGENOUS CYCLE OF *EIMERIA SEPARATA*

Eimeria separata Becker and Hall (1931) parasitizes portions of the epithelial layer of the caecum and colon of the rat. Infection is found to be heavier in the caecum. The cells attacked are found only in the surface epithelium of the caecum and colon, the epithelium of the glands never having been found parasitized. The gametocytes and schizonts are found below the nucleus toward the proximal end of the cell. Some stages, especially macrogametocytes, have been found apparently below the epithelial layer. This apparent location may be because of the plane at which the section was cut, and the parasite may actually be in the base of a cell, the long axis of which was at an angle to the plane of the section.

Becker and Hall (1931) report the prepatent period to be between five and six days. As pointed out for *E. nieschulzi*, this period represents the time elapsed from infection until oöcysts appear in the feces. The endogenous period for this species is four and one-half days.

Sporozoites of *E. separata* may be found in the caecum approximately six hours after infection. Some sporozoites, either because of later excystation or because they fail to enter host cells, have been found free in the lumen of the caecum at three days. This late infection, as in *E. nieschulzi*, complicates the life-cycle and also shows why the patent period should be from three to four days as described by Becker and Hall (1931).

The sporozoites are similar in structure to the sporozoites of *E. nieschulzi*—differing only slightly in cytological composition. They possess an anterior and a posterior refractile globule, both of which are circular in outline. The posterior globule is the larger of the two. Sporozoites have a length range of $7.65\text{--}10.35\mu$, the mean length being 9.45μ . At the nucleus the width varies from $1.8\text{--}2.7\mu$, with a mean of 2.27μ . The nucleus is located at about the middle of the sporozoite and has a central karyosome. The refractile globules are siderophilic and the cytoplasm is granular, the anterior tip being densely granular. (Pl. IV, Fig. 1.)

The sporozoite enters a host cell and forms the first generation schizont. The spherical refractile globules may be found in the early schizont, but disappear during the development. The nucleus divides and the cytoplasm with it until the schizont has the appearance of a morula. Each portion begins to elongate and form a merozoite. This process in no way appears as though the merozoites were budded off of a main body. No residuum has been seen. At about twenty-four hours after infection the first generation merozoites are completely formed and begin to break out of the cell. (Pl. IV, Figs. 2-6.)

These first generation merozoites have a range in length from $10.8\text{--}13.05\mu$, mean length being 11.89μ . They vary in width from $1.8\text{--}2.7\mu$, with an average of 2.25μ . Each group of first generation merozoites contains from 6-12 merozoites. Merozoites of the first generation have a nucleus

which exhibits a central karyosome. The cytoplasm is granular, with a few large granules and, with the exception of the portion immediately surrounding the nucleus takes on even blue stain with iron haematoxylin. The area around the nucleus stains more lightly than the remainder of the cytoplasm. (Pl. IV, Fig. 7.)

The first generation merozoites break out of their host cell, migrate to other cells, and enter them to form the second generation schizont. The early second generation schizont has a large circular area in its center which is not granular at all. The cytoplasm immediately surrounding this area is, in contrast, densely granular.

Schizogony is complete at about 48 hours, and the merozoites formed number from 4-6. These second generation merozoites have a length range from 6.3-9.45 μ , mean 7.74 μ . In width they vary from 1.8-2.7 μ , average being 2.26 μ . The merozoites of this generation are characteristically short and broad. The cytoplasm is stippled and has several large granules scattered throughout. No special designating character except size has been found for this generation. (Pl. IV, Figs. 8-11.)

TABLE 2. *Eimeria separata*. Data concerning the asexual phases

	Sporozoite	Merozoites		
		1st generation	2nd generation	3rd generation
Range	7.65-10.35 μ	10.8-13.05 μ	6.30-9.45 μ	12.6-15.30 μ
Length				
Mean	9.45 μ	11.89 μ	7.74 μ	13.62 μ
Range	1.8-2.7 μ	1.8-2.7 μ	1.8-2.7 μ	2.02-3.15 μ
Width				
Mean	2.27 μ	2.25 μ	2.26 μ	2.63 μ
Range		6-12	4-6	2-6
Number in one schizont				
Mean		8	5.5	4
Time of maturing		1 day	2 days	3 days

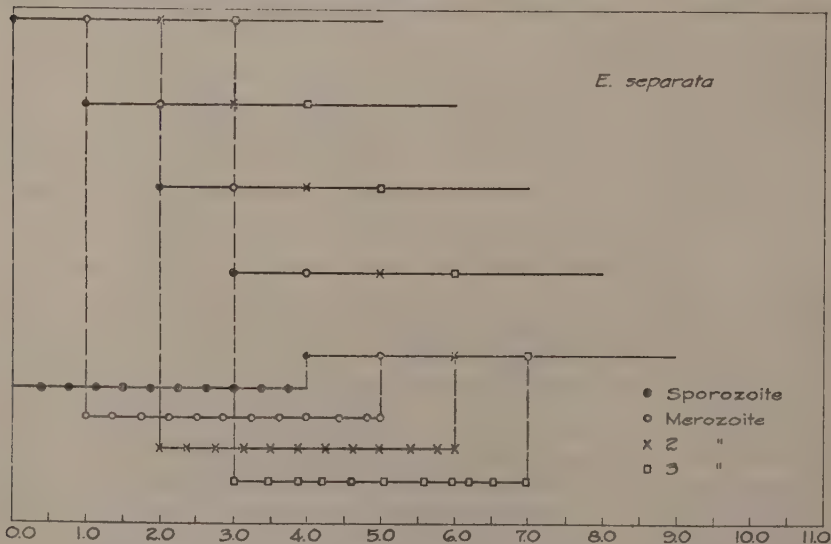
Upon entering other cells, the second generation merozoites form third generation schizonts. The early schizont of this generation looks very similar to other schizonts. The third generation merozoites are mature at about 72 hours after infection. In each group there are 2-6 merozoites formed. These have a length range from 12.6-15.30 μ ; mean, 13.62 μ . In width they vary from 2.02-3.15 μ and average 2.63 μ . This generation not only contains the largest merozoites of this species, but they have one distinguishing character: the tip of the anterior end of each stains an intense red with methylene blue and eosin. With iron haematoxylin this same area stains a rather brownish yellow; the latter color probably is derived from the iron alum used to destain the preparations. No residuum occurs as a result of this schizogony. (Pl. IV, Figs. 12-14.)

Gametocytes are formed when the third generation merozoites infect the epithelial cells. The macro- and the microgametocytes resemble each

other in their early development, but when the nucleus of the microgametocyte divides it can be definitely distinguished. The early gametocytes have an oval vacuolated area which contains an eccentric chromatin mass.

The development of the macrogametocyte parallels that of *E. nieschulzi*, but differs in some details. The granules which go to make up the oöcyst wall arise in the same way, but the haematoxylinophilic ones seem to reach the periphery before the plastic granules. The former do not flatten out, however, until the latter have passed between and have formed the outer oöcyst wall. The haematoxylinophilic granules then flatten out to form the thin inner membrane of the oöcyst. Meanwhile, a second set of plastic granules forms and migrates to just within the inner wall. They remain in this position when the oöcyst is thrown out of the tissues. Their further development has not been traced, but as in *E. nieschulzi* they probably contribute toward the formation of the sporocyst wall at the time of sporulation. (Pl. IV, Figs. 17-19.)

The microgametocyte undergoes its nuclear divisions to form the microgametes without any outstanding variation from *E. nieschulzi*. The chromatin afterwards migrates to the periphery of the cell, begins to elongate, and become comma shaped. Finally, in the last stage it loses its heavy anterior end and becomes rather evenly elongate. This is the mature condition and is the motile phase. These microgametes, because they break out in the caecum and mix with the bacterial content, are rather difficult to find on smear preparations. For this reason the measurements of these gametes are based on specimens found on sections of the caecum. The body of the microgamete varies from 1.8-2.7 μ in length; the width is estimated to be somewhat less than 0.5 μ . (Pl. IV, Figs. 15-16.)



Actual fertilization in this species has not been observed, but since microgametes have been found in the tissues around macrogametocytes,

it is probable that it takes place in the tissues during the early oöcyst wall formation.

The outline of the endogenous cycle indicates that this species has a self-limited cycle. No attempt has been made, however, to check this by transmission of merozoites.

The author does not consider that the fact that *E. separata* has only three generations of merozoites contradicts in any way the data obtained for *E. nieschulzi*. The difference in number of generations is probably only a specific difference, i. e., not generic.

Becker and Hall (1931) observed that the numbers of oöcysts eliminated in an infection of *E. separata* were relatively low. In accordance with this observation several facts should be noted. First, the fact that this species attacks only the surface epithelium of the caecum and colon would tend to make the number of available cells extremely low, as compared with the area attacked by *E. nieschulzi*. Second, the reduced number of generations of merozoites and third, the reduced number of merozoites produced in each generation contribute to considerable reduction in the possible number of gametocytes formed.

To elaborate further on this, multiply the mean number of merozoites formed in each generation by each other and then by the number of sporozoites in one oöcyst and the resultant 1,536 represents the number of gametocytes formed from one oöcyst. The extremes of infection may be found by multiplying the minimum number in each generation by each other and then by eight and by multiplying the maximum numbers by each other and by eight. The range calculated in this manner is from 384 to 3,456 gametocytes per oöcyst fed. These figures not only show the reason for the lowered oöcyst production, but also point out just where the reduction actually takes place.

The prepatent period for *E. separata* is shorter than that for *E. nieschulzi*. The lowered number of generations of merozoites assists in understanding just why this period should be so short.

ENDOGENOUS CYCLE OF *EIMERIA MIYAIRII*

Eimeria miyairii Ohira (1912) passes its endogenous cycle in the epithelium of the mucosa of the small intestine of the rat. (Ohira probably had a mixed culture of this species and *E. separata*. At least, he figured some merozoites of the latter species.) The parasite is found almost exclusively in the epithelium of the villi, but has been found, on a few occasions, down in the glands. One most striking difference between this species and *E. nieschulzi* is the former's habit of causing cells parasitized by it to be pushed out of the epithelial layer toward the tunica propria. This behavior was noted by Ohira in the description of the species, and it is largely upon this basis that the correct determination of the specific name has been made. In addition, this species frequently causes double infections of cells, especially in the later stages of the cycle. (Pl. V, Fig. 12.)

Becker (1934) reported the prepatent period for this species to be six days. The endogenous period is, therefore, about five and one-half days.

Sporozoites of *E. miyairii* are found most abundantly in the small intestine about twelve hours after infection. A series of infections with this species failed to show any sporozoites in the lumen of the small intestine

after two days, but since the time of excystment is so long and since it is probable that they will live in the intestine for four days, the patent period may be accounted for as in the case of *E. nieschulzi*. (See page 138.)

Sporozoites of this species have two refractile globules—one anterior and one posterior to the nucleus. As in the other species, the posterior globule is the larger of the two. The posterior globule is an elongate bar rounded at both ends, while the anterior is circular in outline. The sporozoites have a length range of $12.15\text{--}16.65\mu$; mean, 14.53μ . Width varies from $2.25\text{--}3.15\mu$, with an average of 2.65μ . The nucleus is situated toward the anterior end of the sporozoite, and shows a marginal chromatin ring with a central karyosome. Both refractile globules are siderophilic and the cytoplasm is granular, the anterior end being densely granular. (Pl. V, Fig. 1.)

The sporozoite upon entering a cell forms the first generation schizont. Nothing, as far as has been determined, is distinctive of this schizont. When the first generation merozoites are fully formed a residual mass may be seen in the center with the merozoites arranged in a circle around it. The merozoites leave the host cell in about two days after infection. (Pl. V, Figs. 2-3.)

The first generation merozoites have a range in length from $5.58\text{--}7.20\mu$; mean length, 6.58μ . Width, in these merozoites, varies from $1.08\text{--}1.57\mu$, with a mean of 1.27μ . From 12-24 merozoites are formed from each first schizont. The nucleus of the first generation merozoites is central and has a central karyosome. The cytoplasm is evenly granular, except for a lighter area surrounding the nucleus. (Pl. V, Fig. 4.)

First generation merozoites break out of their host cells and enter other cells to form the second schizont. Since the host cells are situated below the epithelial layer, in most cases, the merozoites get out into the lumen of the intestine by migrating through spaces left by the injured cells. In the cells which do not leave the epithelial layer the parasites are located toward the proximal end of the cell. The second schizonts are, in their early development, similar to other schizonts and so no distinction may be made between them. (Pl. V, Fig. 5.)

Schizogony is completed on the third day of the infection and 8-16 merozoites are formed in each schizont. A residuum is formed in this schizont. The merozoites formed are the second generation merozoites. They have a length range of $8.10\text{--}11.25\mu$; mean length, 9.15μ . Width varies from $1.00\text{--}1.80\mu$, with a mean of 1.44μ . Second generation merozoites are the largest formed in this species. They may be distinguished from other merozoites by the fact that the nucleus is located in the posterior one-fourth of the body. The cytoplasm takes an even stain, except for the area around the nucleus, which, as in other merozoites, takes a lighter stain. These merozoites usually have one or two large granules situated anterior to the nucleus. (Pl. V, Figs. 6-7.)

The second generation merozoites enter cells and form the third generation schizont, which is similar to other schizonts of this species. Thus third generation merozoites formed mature on the fourth day, leaving a large residual body. These merozoites range from 20-24 in number. They have a length range of $3.60\text{--}5.13\mu$, with a mean of 4.37μ ; in width they vary from $1.00\text{--}1.57\mu$, average being 1.23μ . Merozoites of this generation have the usual light area surrounding the nucleus, the remainder of the cytoplasm being evenly granular. (Pl. V, Figs. 8-9.)

Third generation merozoites infect other cells and form the gametocytes. The microgametocytes and macrogametocytes are indistinguishable before the microgametocyte undergoes its first division. These early gametocytes have a central vacuolated area in which an eccentric chromatin mass is located.

TABLE 3. *Eimeria miyairii*. Data concerning the asexual phases

	Sporozoite	Merozoites		
		1st generation	2nd generation	3rd generation
Range	12.15-16.65 μ	5.58-7.20 μ	8.10-11.25 μ	3.60-5.13 μ
Length				
Mean	14.53 μ	6.58 μ	9.15 μ	4.37 μ
Range	2.25-3.15 μ	1.08-1.57 μ	1.00-1.80 μ	1.00-1.57 μ
Width				
Mean	2.65 μ	1.27 μ	1.44 μ	1.23 μ
Range		12-24	8-16	20-24
Number in one schizont				
Mean		18	12	22
Time of maturing		2 days	3 days	4 days

The development of the macrogametocyte differs slightly from the other two species discussed. The plastic granules are extremely large while the haematoxylinophilic granules are very small. The plastic granules migrate to the border of the cell and fuse to form the outer wall of the oöcyst. The fusion is not normally complete and consequently the outer wall of this species is marked with radial striations. In some cases, however, the plastic granules fragment upon reaching the margin of the cell and then fuse. This latter instance, along with the crowded condition of double infections, probably explains the occasional smooth walled oöcysts of this species. When the oöcysts of *E. miyairii* are eliminated from the intestinal wall, the epithelial layer is at least temporarily broken by the passage of the oöcyst through it.

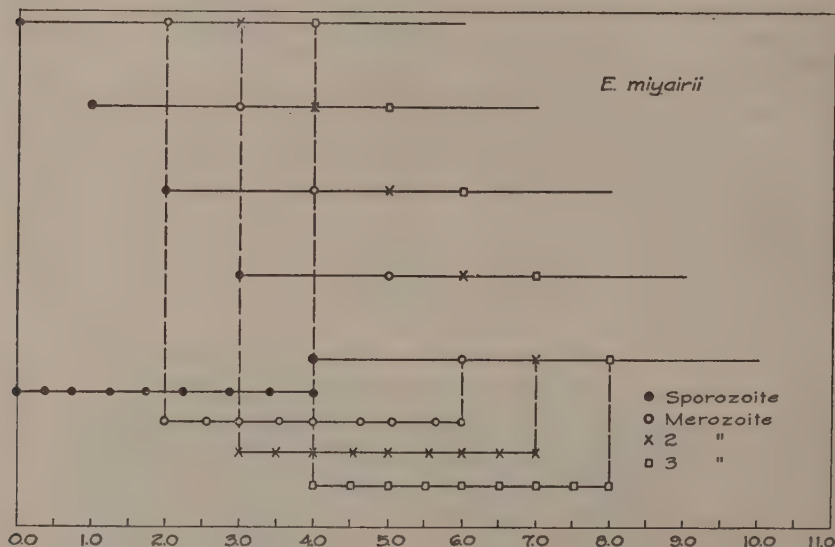
The microgametocyte in its development resembles the microgametocytes of the other species previously described. The chromatin migrates to the periphery of the cell and then elongates to form the microgametes. Typically the microgametes have two flagella attached at the anterior end of the body. The body of the microgametes has a mean length of 3 μ , width being approximately 0.75 μ . A large residual mass is present when the microgametes have reached maturity. (Pl. V, Figs. 10-11.)

Microgametes have been found in the tissues around the macrogametocytes so that fertilization probably takes place while the latter are still in the tissues.

Again it is felt that the endogenous cycle as outlined is indicative of a self-limited life cycle.

Becker (1934) indicated that the number of oöcysts eliminated by a rat infected with *E. miyairii* was not so great as in the case of *E. nieschulzi*,

but greater than in the case of *E. separata*. The present results point toward the same conclusion. To compare this with the other two species, the mean number of merozoites in each generation should be multiplied by each other, and then by the number of sporozoites in one oöcyst. This calculation gives a mean number of 38,016 gametocytes formed per oöcyst fed. The extremes of infection are gotten by multiplying the extremes of the numbers of merozoites by each other and then by eight. This gives a minimum number of 25,360 and a maximum of 73,728 gametocytes per oöcyst fed. These figures show the possible extremes and the average infection.



The double and triple infections in single cells by *E. miyairii* are interesting in view of the recent work by Beach (1936) on *Plasmodium vivax*. He found that multiple infections probably arose by the division of the schizont after infecting the cells. This is not true for the *Eimeria* under consideration. It has been shown that each macrogametocyte is formed from a third generation merozoite and each third generation schizont is formed from a second generation merozoite. Since many of the doubly infected cells contain both macrogametocytes and third generation schizonts it is evident that the infecting form for each differed, and that they were not formed by the division of one merozoite after entering the host cell.

SUMMARY AND CONCLUSIONS

The endogenous cycles of the three species of *Eimeria* studied indicate several important principles concerning infection with coccidian parasites. First, as emphasized before, the fact that the cycles are definite and exact indicates that they are limited not by the host but by the parasites themselves. Second, it would appear that no sexual dimorphism is present in the asexual phases. The merozoites heretofore described by

Dieben (1924), Hosoda (1929) and others as sexual dimorphic forms are probably different generations of merozoites. Third, this study shows that localization of infection is characteristic for each species. Fourth, each species has an individualistic life history which need not be exactly like that of any other coccidium.

TABLE 4. Comparison of the three species of coccidia in rats

	<i>E. nieschulzi</i>	<i>E. separata</i>	<i>E. miyairii</i>
Name applied by Becker 1934	<i>E. miyairii</i>	<i>E. separata</i>	<i>E. carinii</i>
*Range in size of oöcyst	16.2-26.4 μ x 13.4-21.3 μ	12.8-19.4 μ x 11.2-17.2 μ	16.8-29 μ x 16.1-26 μ
*Mean size of oöcyst	22.5 μ x 17.8 μ	16.06 μ x 13.85 μ	24.38 μ x 22.12 μ
*Character of the wall	Smooth or granular	Smooth	Radial striated, rough
*Prepatent period	7-8 days	5-6 days	6 days
Endogenous period	6.5 days	4.5 days	5.5 days
Number of merozoite generations	4	3	3
Part of intestine affected	Small intestine	Caecum and colon	Small intestine

* Data taken from Becker 1934 with permission of the Collegiate Press, Ames, Iowa.

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PLATE I

Eimeria nieschulzi

All drawings made with the aid of camera Lucida. Unless otherwise stated all motile phases are stained with iron haematoxylin, those in cells with methylene blue and eosin. All figures x 2222.

1. Sporozoite.
2. Sporozoite with divided posterior globule.
3. Sporozoite after entering host cell.
4. First generation merozoites in cell; merozoites shown in cross section. Note the refractile globule.
5. Group of first generation merozoites.
6. First generation merozoite.
7. First generation merozoite after entering host cell. (Iron haematoxylin.)
8. Second generation schizont.
9. First nuclear division in second schizont.
10. Group of second generation merozoites.
11. Second generation merozoite.

PLATE I

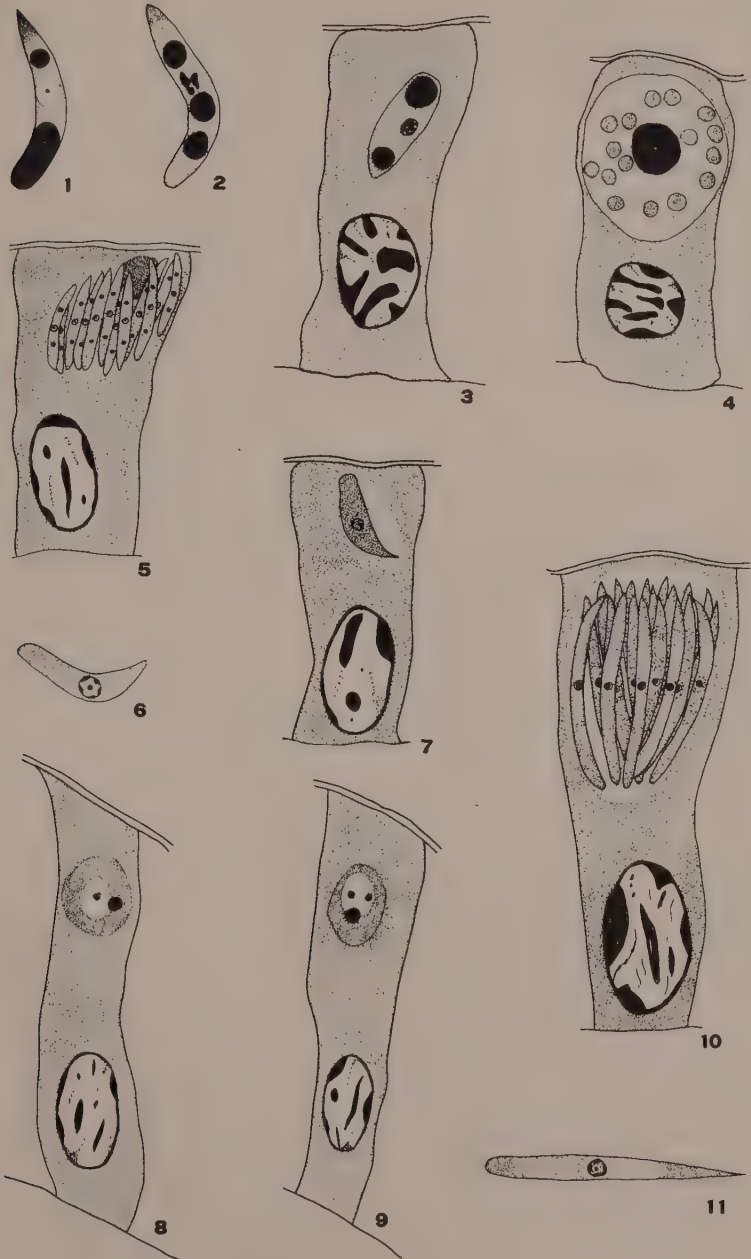


PLATE II

Eimeria nieschulzi

1. Group of third generation merozoites.
2. Third generation merozoite.
3. Division in fourth schizont.
4. Division in fourth schizont.
5. Group of fourth generation merozoites.
6. Fourth generation merozoite.
7. First division in micromagetocyte.
8. Later stage in microgamete development.
9. Microgametocyte (surface view).
10. Microgamete.
11. Early macrogametocyte.
12. Mature macrogametocyte (oöcyst).

PLATE II

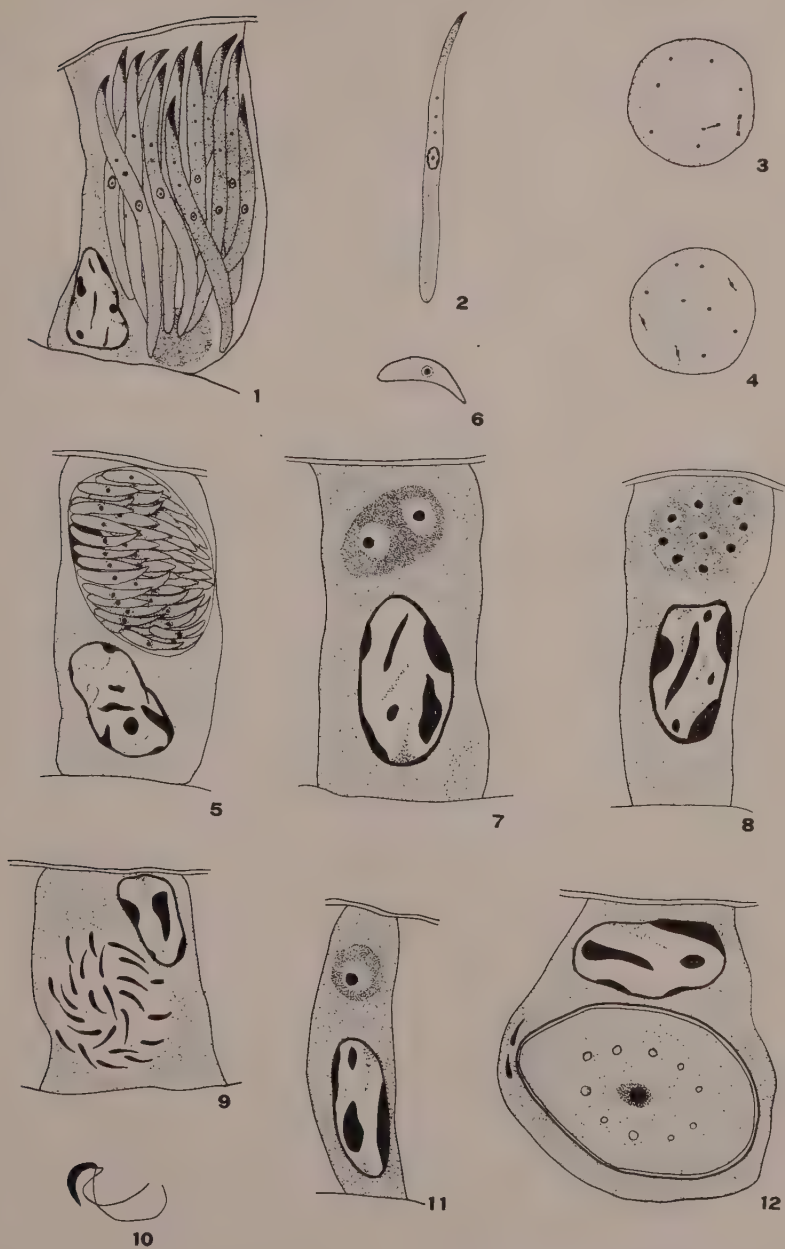


PLATE III

Eimeria nieschulzi

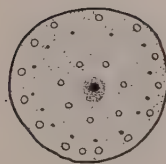
Deve'opment of the oöcyst wall. Plastic granules shown as white bodies with a solid outline. Haematoxylinophilic granules shown as large black dots.

1. Early development—showing migration of granules.
2. Plastic granules have almost reached the periphery. The second group of plastic granules remains close to the nucleus.
3. Plastic granules have reached the edge and begin to flatten out. Haematoxylinophilic granules begin to elongate.
4. Outer wall formed from the plastic granules. Inner wall forming from the haematoxylinophilic granules.
5. Inner wall almost fully formed.
6. Mature oöcyst inner wall shrunken away from the outer because of dehydration. Second set of plastic granules shown in the cytoplasm.

PLATE III



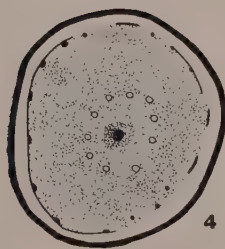
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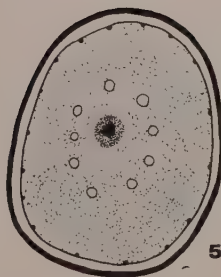
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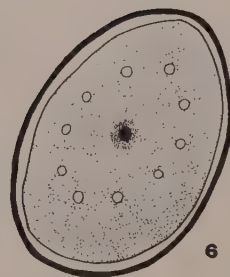
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6

PLATE IV

Eimeria separata

1. Sporozoite.
2. Sporozoite in host cell.
3. Sporozoite after rounding up.
4. First division in first schizont.
5. Early first generation merozoites.
6. Group of first generation merozoites.
7. First generation merozoite.
8. First generation merozoite after entering cell.
9. Early second schizont (double infection).
10. Group of second generation merozoites.
11. Second generation merozoites.
12. Group of third generation merozoites.
13. Third generation merozoite.
14. Third generation merozoite after entering cell.
15. Microgametocyte (surface view).
16. Microgamete.
17. Young macrogametocyte.
18. Later macrogametocyte.
19. Almost mature macrogametocyte.

PLATE IV

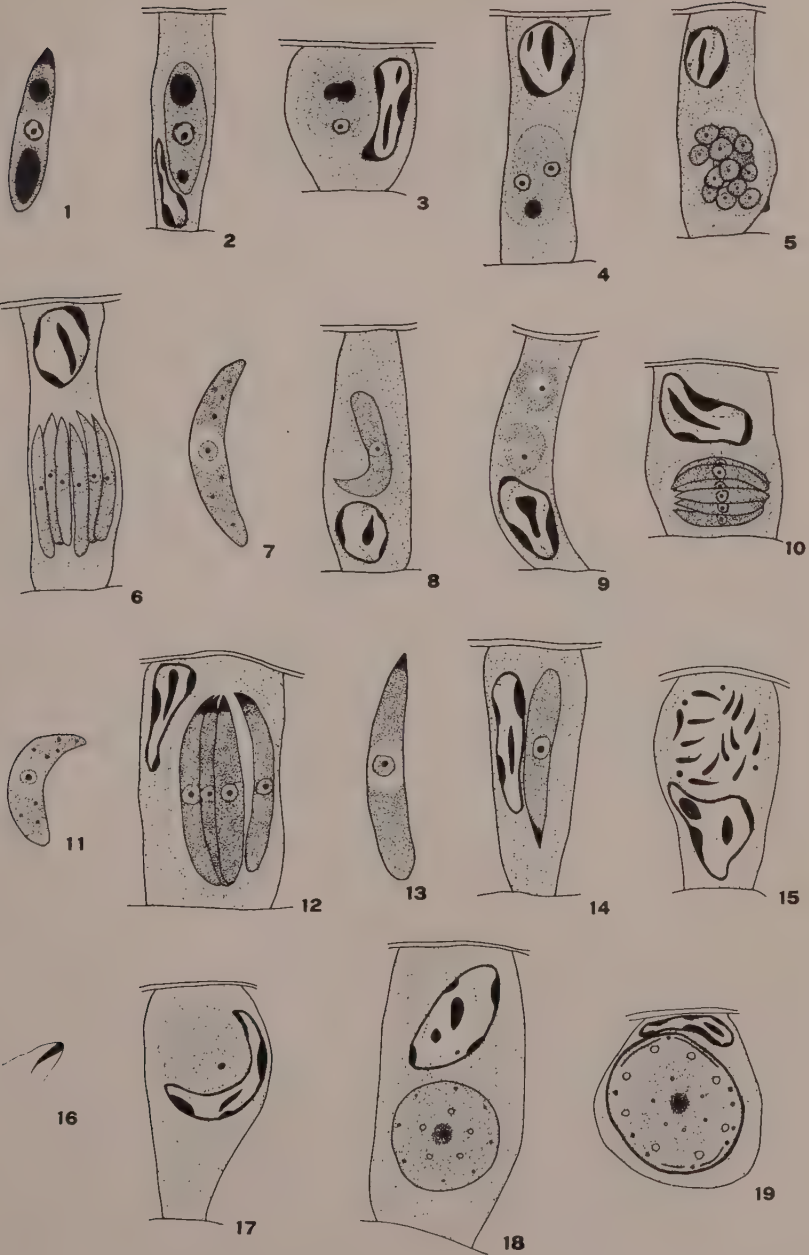
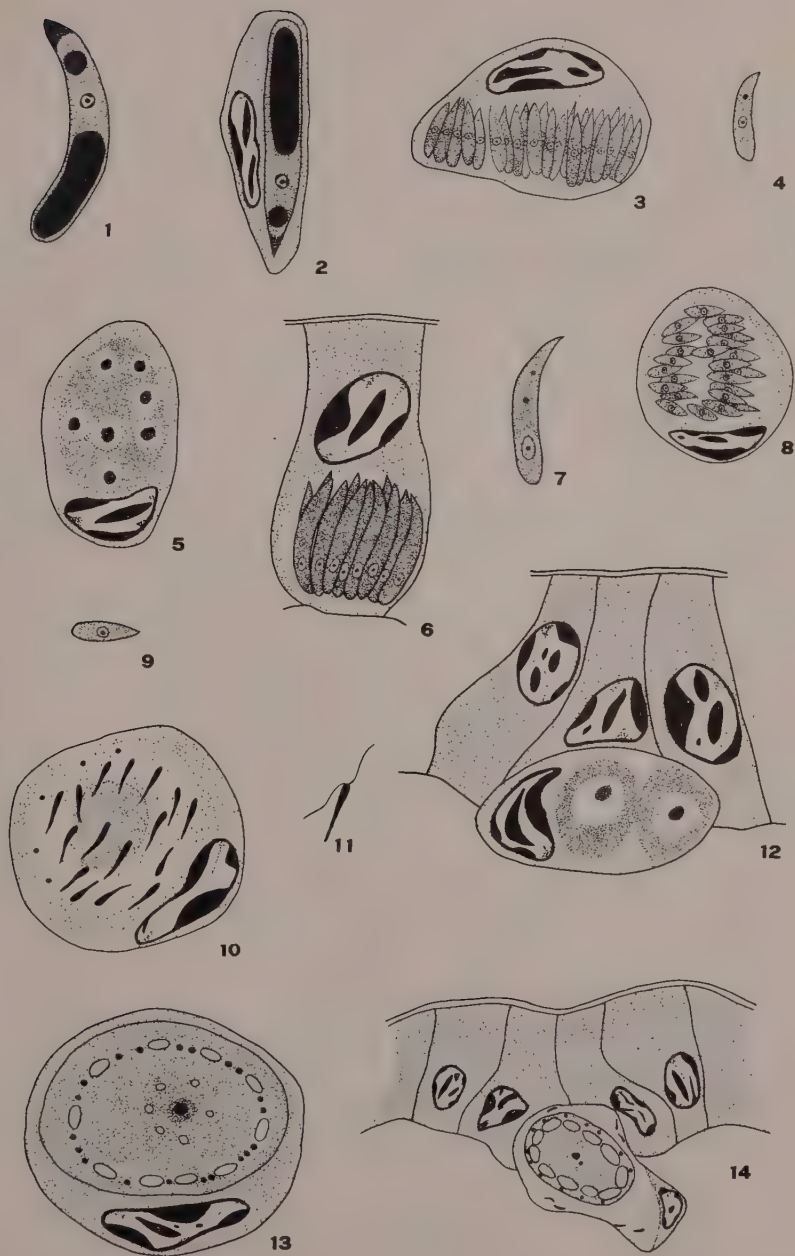


PLATE V

Eimeria miyairii

1. Sporozoite.
2. Sporozoite in host cell.
3. Group of first generation merozoites.
4. First merozoite.
5. Schizont (probably second generation).
6. Group of second generation merozoites.
7. Second generation merozoite.
8. Group of third generation merozoites.
9. Third generation merozoite.
10. Microgametocyte (surface view).
11. Microgamete.
12. Double infection, with young macrogametocytes shown below epithelium.
13. Macrogametocyte.
14. Figure copied from Ohira (1912) showing typical macrogametocyte.

PLATE V



AN UNDESCRIBED CHINCH BUG FROM IOWA¹

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During the past several years chinch bugs have caused tremendous losses of crops in Iowa. Rather intensive studies as to their ecological, biological and systematical relationships have been made. For these studies collections have been taken in every county in the state. Most of the specimens, however, were collected in southern Iowa, where heaviest losses from the infestation have occurred. The present paper deals with the systematic phase of the chinch bug problem.

In connection with hibernation studies on chinch bugs made during the past winter, some samples³ of little blue stem (*Andropogon furcatus* Muhl.) were obtained near Creston, Union County, Iowa. These samples not only contained numerous specimens of the common chinch bug (*Blissus leucopterus* Say), but they also showed the presence of another species quite distinct from *B. leucopterus*. At first glance, this was thought to be the northwest chinch bug (*Blissus occiduus* Barber), a species which has been found in several localities in the western states and Canada. A careful study of these specimens, however, revealed that they were structurally different and could be readily separated from specimens of *Blissus occiduus* collected in Colorado, Montana and in Saskatchewan, Canada. The species, therefore, is described herein as new to science.

BLISSUS IOWENSIS n. sp. (Plate I)

Holotype (brachypterous ♂). Length 2.80 mm.; width at widest portion of abdomen 0.81 mm. General color black. Legs, segment II of antennae, and rostrum ochraceous. Eyes deep red. Ocelli red. Wing bracts milkish white. Head pronotum dorsal and ventral portions of abdomen covered with pale incumbent hairs.

Head much deflexed, rather short and broad. Eyes moderately prominent. Hairs on tylus longest. Width across eyes 0.58 mm. Antennae inserted ventral to eyes; segment IV longest, slightly more than twice as long as III; I shortest about half as long as II; segments in the proportions as I:II:III:IV::12:23:18:38. Segments II, III and IV distinctly club-shaped. Segment I, ochraceous; II, light yellow shading to ochraceous at apex; III, ochraceous in basal third shaded to piceous in apical two-thirds, in some examples entirely piceous. Rostrum four-segmented reaching past hind margin of posterior coxae; segment II, longest about 1.5 as long as III, which is shortest; segments I and IV about equal in lengths; seg-

¹ Journal Paper No. J373 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 420.

² The writer wishes to express his appreciation to Miss M. E. Poor for making the plate, and to Dr. C. J. Drake for many useful suggestions.

³ These were obtained from A. D. Worthington of the Iowa Agricultural Extension Service.

ments in the proportions as I:II:III:IV::22:30:20:22. All segments ochraceous, I slightly darkened at base and IV darkened at apex.

Pronotum wider than long (81:53), strongly transverse, lateral margins parallel to each other in posterior half, prominently punctured, covered with rather erect pale pubescence. Scutellum twice as wide as long (42:21), impunctate, covered with fine pubescence. Hemelytra short, reaching to posterior margin of metasternum. Membrane much reduced, pale milky white, apical margin rounded, reaching posterior margin of first abdominal segment. Dorsum of abdomen covered with many pale incumbent hairs, with a few more erect hairs on sides. Legs with femora brownish; tibia yellowish with brownish area on knee; tarsi yellowish-brown; claws yellow. Venter black with pale incumbent pubescence; genital claspers pale yellow, somewhat smaller than in *leucopterus*.

Allotype (*Brachypterus* ♀). Similar to male in color and structure, slightly larger. Length 3.1 mm.; width at widest portion of abdomen 0.92 mm.

Macropterous forms (♂ and ♀). Lateral margins of pronotum parallel to each other posteriorly. Corium reaching to posterior margin of the third abdominal segment. Veins slightly tinged with brown, remainder of wing pale milky white. Membrane in examples examined never reaching to tip of abdomen.

Type locality: Creston, Iowa.

Described from brachypterous male *holotype* and brachypterous female *allotype* in the collection of Iowa State College. *Paratypes*: 4 macropterous males, 3 macropterous females, 30 brachypterous males, and 34 brachypterous female paratypes all taken with type overwintering in little blue stem, *Andropogon furcatus*, at Creston, Iowa, during March, April and May, 1936. Paratypes have been deposited in the United States National Museum (Washington, D. C.), British Museum (London), Stockholm Museum and Iowa State College.

B. iowensis may be distinguished from *occiduus*, its closest American relative, by the more club-shaped antennal segments, the more projecting eyes, the relatively longer terminal antennal segment, and the relatively longer second rostral segment. From typical *leucopterus* and its varieties it may readily be separated by its smaller size, the more flattened pronotum, the veins of the fore wings and the relatively longer rostrum, which in *iowensis* reaches past the hind margin of the posterior coxae.

After studying a long series of specimens of *B. occiduus* from various localities in the west the writer feels that *iowensis* should be treated as a distinct species rather than as a variety of *occiduus*. The distribution of *iowensis* is interesting in that although careful search has been made for it in numerous localities, all the specimens of it so far have come from Union County, Iowa. In a subsequent paper the biology and habits of *iowensis* will be reported by Dr. G. C. Decker and the writer. It might be mentioned that the rearing experiments indicate that *iowensis* feeds and apparently thrives on little blue stem. It has not been found feeding on any cultivated crop under field conditions.

PLATE I

Drawing of male holotype of *Blissus iowensis* n. sp.

PLATE I



CONTRIBUTIONS TO THE SOUTH DAKOTA LIST OF HEMIPTERA

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Through the courtesy of Professor H. C. Severin, it has been my privilege to study the miscellaneous Hemiptera added to the collection of the South Dakota State College during the past ten years. There are represented in the collection most of the species recorded by Dr. H. M. Parshley in his preliminary list¹ and in addition a number of forms not heretofore recorded from the state. It is with the latter that this report is chiefly concerned. However, in order to make the list as inclusive as possible, data on certain species belonging in the various collections at Ames are given as also are the names of the species recorded by Parshley. The Miridae, representing in number of specimens almost half of the material, have been turned over to Dr. H. H. Knight and will be reported on by him in a separate paper. The Corixidae are as yet unnamed.

In Parshley's report there are recorded as occurring in South Dakota 162 species, of which 50 are Miridae. To the 112 species belonging to families other than Miridae may now be added 62 additional forms. In the following pages species recorded by Parshley but not represented in the material before me are listed without locality data. Forms not recorded by Parshley, that is, additions to his list, are indicated with the asterisk. From the standpoint of a state list it would seem desirable to present every different date and locality record for each species. Because of limitations of publication space, however, such has not been possible in this paper. Usually only the first and latest seasonal records are presented. The names of the collectors, H. C. Severin, G. I. Gilbertson, F. R. Bingham and G. B. Spawn, are indicated by their initials.

Family SCUTELLERIDAE

Homaemus aenifrons (Say). Deadwood, 9-ix-'25, H. C. S.

Homaemus bijugis Uhler. Springfield, 16-vi-'26, H. C. S.; Wewela, 16-ix-'30, H. C. S.

Eurygaster alternatus (Say)*. Chester, 16-vi-'33, F. R. B.; Hecla, 30-viii-'27, H. C. S.; Cave Hills, 22-vii-'28, H. C. S.; Lakeview, 20-vi-'28, G. I. G.

Phimodera binotata (Say)*. Forestburg, 21-viii-'29, H. C. S.; Sand Hills, Martin, 25-viii-'29, G. I. G.

Family CYDNIDAE

Pangaeus bilineatus (Say)*. Elk Point, 25-viii-'26, H. C. S.; Springfield, 16-vi-'26, H. C. S.

¹Parshley, H. M. Report on a collection of Hemiptera-Heteroptera from South Dakota. S. D. State College, Tech. Bul. (2):1-22, figs. 2. 1922.

Cydnus obliquus (Uhl)*. Hecla, 17-vi-'34, H. C. S.; Chester, 22-vii-'29, G. B. S.

Homaloporus congruus Uhl*. Buffalo Gap, 23-vi-'32, F. R. B.

Sehirus cinctus (P. B.). Browns Valley, 23-vi-'27, H. C. S.

Family THYREOCORIDAE

Galgupha nitiduloides (Wolf). Englewood, 18-vi-'25, G. I. G.; White River, 21-vi-'31, H. C. S.; Elk Point, 13-vi-'26, G. I. G. Recorded by Parshley without data.

Galgupha atra A. & S.

Allocoris pulicaria (Germar). Brookings, 14-vi-'31, G. B. S.

Allocoris nigra (Dallas). Hecla, 19-vi-'33, H. C. S.

Allocoris gillettii (Van Duzee). The *lateralis* of previous lists.

Allocoris extensa (Uhler)*. Lead, 18-vi-'34, H. C. S.

Family PENTATOMIDAE

Brochymena quadripustulata (Fabr.). Vermillion, 26-vi-'35, H. C. S.

Peribalus limbolarius Stål. Hecla, 19-vi-'30, H. C. S.

Peribalus piceus (Dallas).

Peribalus abbreviatus Uhler*. Buffalo, 19-vi-'25 and 8-ix-'25, H. C. S.

Rhytidolomia faceta Say. Eureka, 5-ix-'30; Selby, 20-vii-'27, H. C. S.

Chlorochroa uhleri Stål. Englewood, 18-vi-'25, H. C. S.; Wewela, 16-ix-'30, H. C. S.

Chlorochroa ligata (Say)*. Custer, 20-vi-'34 and 4-viii-'33, H. C. S.; Hecla, 19-vi-'33, H. C. S.

Carpocoris remotus Horvath*. Buffalo, 3-viii-'35, H. C. S.; Slim Buttes, 13-viii-'35, H. C. S.

Euschistus latimarginatus Zimmer* Buffalo, 19-vi-'25 and 9-ix-'27, H. C. S.

Euschistus ictericus Linn.* Sand Hills, Martin, 15-ix-'30, H. C. S.

Euschistus euschistoides (Vollenhoven). Englewood, 21-viii-'33, H. C. S.; Elk Point, 12-vi-'25, H. C. S.; Springfield, 14-ix-'30, H. C. S.

Euschistus tristigmus (Say). Warren Woods, White, 25-ix-'25, H. C. S.

Euschistus variolarius (P. B.). Jefferson, 25-viii-'32, H. C. S.; Canton, 12-vi-'26, H. C. S.

Coenus delius (Say). Jefferson, 25-viii-'32, H. C. S.

Hymenarcys aequalis (Say)*. Brookings, 3-viii-'28.

Hymenarcys nervosa (Say).

Aelia americana Dallas*. Tripp, 15-vi-'21, H. C. S.; Medicine Lake, Florence, 23-vi-'27, H. C. S.

Neottiglossa undata (Say). Custer, 31-vii-'32, F. R. B.

Neottiglossa sulcifrons Stål. Springfield, 24-vi-'34, H. C. S.

Cosmopepla bimaculata (Thomas). Englewood, 21-viii-'33, H. C. S.

Menecles incertus (Say)*. Springfield, 25-vi-'35 and 8-ix-'34, H. C. S.

- Thyanta custator* (Fabr.) Philipp, 16-ix-'32, H. C. S.
Thyanta brevis V. D.
Thyanta rugulosa Say*. Pierre, 5-ix-'26, H. C. S.
Murgantia histrionica (Hahn).
Acrosternum hilare (Say).
Banasa dimidiata (Say). Brookings, 4-viii-'22, H. C. S.
Perillus bioculatus (Fabr.). Onida, 15-vii-'33, G. I. G.
Perillus bioculatus clanda (Say). Custer, 21-vii-'33, F. R. B.
Perillus exaptus (Say)*. Waubay, 29-viii-'27, H. C. S.
Apateticus bracteatus (Fitch).
Podisus maculiventris (Say). Brookings, 3-viii-'28, Fredricksen.
Podisus placidus Uhler*. Elk Point, 12-vi-'25, H. C. S.; Bonesteel, 26-viii-'29, H. C. S.
Podisus modestus (Dallas)*. Englewood, 18-vi-'25, H. C. S.

Family COREIDAE

- Merocoris distinctus* Dallas. Springfield, 14-vi-'25, H. C. S.; White, 15-ix-'25, H. C. S.
Acanthocephala terminalis (Dallas)*. Yankton, 18-viii-'27, H. C. S.; Springfield, 26-viii-'29 and 18-viii-'33, H. C. S.
Leptoglossus occidentalis Heidemann.
Leptoglossus clypealis Heidemann*. Hot Springs, 30-viii-'32, F. R. B.; Onida, 25-vi-'32, G. B. S.
Archimerus alternatus (Say). Iona, 25-vi-'31, H. C. S.
Euthoctha galeator (Fabr.). Yankton, 5-ix-'28, H. C. S.; Springfield, 16-vi-'26, H. C. S.
Chariesterus antennator (Fabr.). Elk Point, 24-vi-'26, H. C. S.; Springfield, 14-ix-'25, H. C. S.
Chelinidea vittiger Uhler*. Milesville, 23-vi-'31, H. C. S.; Rosebud, 17-vi-'36, G. I. G.
Catorhintha mendica Stål. Browns Valley, 23-vi-'27, H. C. S.; Brookings, 1-x-'31, H. C. S.
Anasa armigera (Say)*. Brookings, 10-vii-'31, G. B. S.
Anasa tristis (DeGeer). Capa, 15-viii-'22, H. C. S.
Coriomeris humilis Uhler. Buffalo, 19-vi-'25, H. C. S.; Pringle, 14-x-'31, H. C. S.

Family CORISCIDAE

- Protenor belfragii* Haglund. Brookings, 8-viii-'28, H. C. S.; 11-ix-'34, H. C. S.
Darmistus subvittatus Stål*. Cascade Springs, 30-vii-'35, H. C. S.
Megalotomus quinquespinosus (Say). White, 10-viii-'27, H. C. S.; Elk Point, 15-ix-'25, H. C. S.
Coriscus eurinus obesus (Fracker). Springfield, 17-ix-'30, H. C. S.

- Coriscus eurinus eurinus* (Say) *. Elk Point, 15-ix-'25, H. C. S.
Coriscus pluto (Uhler) *. Provo, 20-vi-'28, H. C. S.; Cave Hills, 22-vii-'28, H. C. S.
Coriscus conspersus (Montandon). Chamberlin, 15-vi-'28, H. C. S.; Wau-bay, 13-ix-'29, H. C. S.
Coriscus conspersus infuscatus (Fracker) *. Onida, 18-vi-'33, H. C. S.
Coriscus pilosulus (H. S.). Hot Springs, 12-ix-'27, H. C. S.
Stachyocnemus apicalis (Dallas) *. Elk Point, 15-ix-'25, H. C. S.

Family CORIZIDAE

- Harmostes reflexus* (Say). Canton, 12-vi-'26, H. C. S.; Martin, 12-ix-'25, H. C. S.
Aufeius impressicollis Stål. Grass Rope, 21-vi-'34, H. C. S.; Brookings, 1-x-'31, H. C. S.
Corizus punctiventris (Dallas). Englewood, 18-vi-'25, H. C. S.; Dead-wood, 9-ix-'25, H. C. S. The *crassicornis* Auct., nec Linn.
Corizus viridicatus Uhler. Belle Fourche, 24-vi-'28, H. C. S. The true *viridicatus* of Uhler.
Corizus lateralis Say. Canton, 12-vi-'26, H. C. S.; Springfield, 14-x-'25, H. C. S.
Corizus sp.* Lead, 2-viii-'35, H. C. S. This is a form usually confounded with *scutatus* Stål and *identatus* Hambleton. It will be discussed in a forthcoming paper on the genus.
Corizus hyalinus (Fabr.)*. Gregory, 18-viii-'31, H. C. S.; Lead, 2-viii-'35, H. C. S.
Leptocoris trivittatus (Say). Springfield, 18-vi-'30, G. I. G.
Jadera haematoloma (H. S.)*. Elk Point, 12-vi-'24, H. C. S.; Lake Hen-dricks, 28-vi-'25, H. C. S.

Family NEIDIDAE

- Neides muticus* (Say). Englewood, 18-vi-'25, H. C. S.; Warrenwoods, White, 25-ix-'25, H. C. S.
Jalysus spinosus (Say). Canton, 12-vi-'26, H. C. S.; Springfield, 14-ix-'25, H. C. S.

Family LYGAEIDAE

- Oncopeltus fasciatus* (Dallas).
Lygaeus turcicus Fabricius.
Lygaeus kalmii Stål. Buffalo, 11-ix-'34, H. C. S.
Lygaeus kalmii angustomarginatus Parshley. Englewood, 18-vi-'25, H. C. S.
Lygaeus bistrigularis Say*. Pringle, 14-ix-'30, H. C. S.
Lygaeus pusio (Stål).
Lygaeus bicrucis Say*. Brookings, 3-viii-'25, H. C. S.
Lygaeus lateralis Dallas*. Englewood, 18-vi-'25, H. C. S.

- Lygaeus tripunctatus* (Dallas) *. Pringle, 14-ix-'30, H. C. S.
Ortholomus scolopax (Say) *. Martin, 12-ix-'25, H. C. S.
Nysius californicus Stål. Gregory, 18-vi-'31, H. C. S.
Nysius ericae (Schilling). Onida, 18-vi-'31, H. C. S.; Englewood, 14-ix-'30, H. C. S.
Ischnorrhynchus resedae Panzer. Brookings, 7-ix-'29, H. C. S.
Cymus luridus Stål. Deadwood, 9-ix-'25, H. C. S.
Ischnodemus falicus (Say). Springfield, 25-vi-'35, H. C. S.
Ischnodemus hesperius Parshley. Belle Fourche, 21-vii-'28, H. C. S. The types were from Brookings.
Blissus leucopterus (Say).
Geocoris bullatus (Say). Platte, 16-vi-'33, H. C. S.
Geocoris pallens decoratus Uhler.
Geocoris uliginosus limbatus Stål.
Oedancala dorsalis (Say). Springfield, 16-vi-'26, H. C. S.
Sphaerobius insignis (Uhler). Buffalo, 20-vi-'25, H. C. S.
Ligyrocoris diffusus (Uhler). Springfield, 16-vi-'26, H. C. S.; Custer, 1-ix-'26.
Ligyrocoris sylvestris (Linn.) *. Custer, 29-vii-'35, H. C. S.
Perigenes constrictus (Say). Chester, 26-vii-'30, G. B. S.
Zeridoneus costalis (V. D.)
Pseudocnemodius canadensis (Prov.)
Plinthisus compactus (Uhler) *. Brookings, 28-x-'29, P. H. Johnston. A single specimen that is identical with individuals from New York.
Peritrechus fraternus Uhler. Chester, 28-vii-'30, G. B. S.
Sphragistus nebulosus (Fallen). Vivian, 19-vi-'28, H. C. S.
Aphanus umbrosus (Distant) *. Bonesteel, 26-viii-'29, H. C. S.; Chester, 12-vii-'31, H. C. S.
Uhleriola floralis (Uhler).
Emblethis vicarius Horvath. Brookings, 1-v-'27, H. C. S.

Family PIESMIDAE

- Piesma cinerea* Say. Belle Fourche, 24-vi-'28, H. C. S.
Piesma cinerea inornata McAtee.

Family TINGIDAE

- Gargaphia tiliae* (Walsh). Newton Hills, Canton, 12-vi-'36, H. C. S.
Leptostyla oblonga (Say).
Corythucha ulmi O. & D.
Corythucha distincta O. & D.
Corythucha marmorata (Uhler). Elk Point, 25-viii-'26, H. C. S.
Corythucha marmorata informis Parshley.
Corythucha arcuata (Say). Canton, 16-viii-'27, H. C. S.

Corythucha arcuata mali Gibson.

Leptoypha mutica Say.

Dolichocysta venustata Champion*. (= magna Gibson.) Hill City.

Family PHYMATIDAE

Phymata erosa fasciata (Gray). Martin, 5-ix-'31, H. C. S.; Buffalo, 22-vii-'28, H. C. S. (= *P. americana* Melin?)

Family REDUVIIDAE

Metapterus uhleri (Banks).

Metapterus uhleri brunnea (Banks).

Reduvius personatus (Linn.). Brookings, 8-vii-'35, H. C. S.

Melanolestes picipes (H. S.)*. Brookings, 10-i-'31, H. C. S.

Melanolestes picipes abdominalis (H. S.).

Apiomerus spissipes (Say). Milesville, 23-vi-'31, H. C. S.

Zelus socius Uhler*. Milesville, 23-vi-'31, H. C. S.

Rhynocoris ventralis (Say)*. Oelrichs, 22-vi-'30, G. I. G.; Buffalo, 20-vi-'25, H. C. S.; Custer, 29-vii-'35, H. C. S.

Fitchia aptera Stål. Fox Ridge, 22-vi-'27, H. C. S.

Acholla multispinosa (DeGeer)*. Yankton, 18-viii-'27, H. C. S.; Elk Point, 25-viii-'26, H. C. S.

Sinea diadema (Fabr.). Elk Point, 12-vi-'25, H. C. S.; Chester, 13-ix-'30, G. B. S.

Sinea spinipes (H. S.)*. Elk Point, 25-viii-'26, H. C. S.

Family NABIDAE

Pagasa fusca (Stein)*. East Sioux Falls, 12-vi-'29, H. C. S. Nymphs of various instars, as well as adults.

Nabis vanduzeei (Kirk)*. Cave Hills, 22-vii-'28, H. C. S.

Nabis subcoleoptratus (Kirby). Gregory, 24-vi-'31, H. C. S. The rare macropterus form is represented by two females.

Nabis ferus (Linn.). Brookings, 22-vii-'35, A. G. Peterson.

Nabis ferus pallidipennis Harris*. Clear Lake, 15-vii-'30, R. C. Bushland.

Nabis alternatus Parshley. Custer, 3-vii-'32, F. R. B. The holotype was from Capa.

Nabis rufusculus Reuter. 11-ix-'34, H. C. S.

Nabis roseipennis Reuter. Lake Hendricks, 25-ix-'25, H. C. S.

Family CIMICIDAE

Cimex lectularius Linn. Hot Springs, 27-viii-'22, H. C. S.

Family ANTHOCORIDAE

Anthocoris borealis Dallas.

Orius insidiosus (Say). Elk Point, 17-vii-'33, H. C. S.

Orius tristicolor (White). Deadwood, 9-ix-'25, H. C. S.

Lyctocoris n. sp.* Black Hills. Taken on *Pinus ponderosa*. This form is to be reported on elsewhere.

Family MIRIDAE

Dr. Parshley's list included 50 names in this group, and the material to be reported on by Dr. Knight should add many more.

Family GERRIDAE

Gerris remigis Say*. Newton Hills, Canton, 24-vi-'35, H. C. S.

Gerris nyctalis Drake & Hottes*. Custer, 11-ix-'34, H. C. S.; Englewood, 21-viii-'33, H. C. S.; Deadwood, 9-ix-'25, H. C. S.; Hot Springs, 17-ix-'32, F. R. B.

Gerris marginatus Say. Springfield, 15-vi-'28, H. C. S.

Gerris comatus Drake & Hottes*. Brookings, 4-vi-'28, H. C. S.; Springfield, 15-vi-'28, H. C. S.; Sand Hills, Martin, 15-ix-'30, H. C. S.

Gerris buenoi Kirkaldy. Brookings, 4-vi-'28, H. C. S.

Gerris dissortis Drake and Harris. The *rufoscutellatus* Auct. nec Latr.

Metrobates hesperius Uhler. Hudson, 24-vii-'32, H. C. S.

Family HEBRIDAE

Merragata foveata Drake*. Brookings, 28-v-'22, H. C. S.; Lake Hendricks, 12-vii-'22, H. C. S.

Family MESOVELIIDAE

Mesovelia bisignata Uhler. Recorded in literature as *mul santi* White.

Family VELIIDAE

Microvelia americana (Uhler)*. Deadwood, 9-ix-'25, H. C. S.; Cascade Springs, 30-vii-'35, H. C. S.

Rhagovelia oriander Parshley. Brookings is the type locality.

Family SALDIDAE

Saldula major (Prov.)*. Newton Hills, Canton, 24-vi-'35, H. C. S.

Saldula interstitialis (Say)*. Lake Poinsett, 29-viii-'27, H. C. S.; Sand Hills Batesland, 20-vi-'28, H. C. S.

Saldula comatula Parshley*. Sand Hills, Batesland, 20-vi-'28, H. C. S.

Saldula xanthochila (Fieb.)*. Lake Poinsett, 29-viii-'27, H. C. S.

Saldula n. sp.* Waubay, 22-vi-'36, H. C. S.

Salda obscura Prov.*. Englewood, 18-vi-'25, G. I. G.

Salda coriacea (Uhl.)*. Lake Oakwood, 28-vi-'29, G. I. G.

Micracantha humilis (Say)*. Lake Poinsett, 29-viii-'27, H. C. S.

Family NOTONECTIDAE

Notenecta kirbyi Hungerford. This is the form recorded by Dr. Parshley as *insulata*.

Notonecta irrorata Uhler.

Notonecta undulata Say. Brookings, 9-viii-'31, H. C. S.

Buenoa margaritacea Torre-Bueno.

Family NAUCORIDAE

Pelocoris femoratus P. B.

Ambrysus heidemanii Montandon. Cascade Springs, 30-vii-'35, H. C. S.

These specimens are inseparable from an individual in my collection from Yellowstone, the type locality of *heidemanni*.

Family NEPIDAE

Ranatra americana Montandon.

Ranatra kirkaldyi Torre-Bueno.

Ranatra protensa Montandon.

Family BELOSTOMATIDAE

Benacus griseus (Say) *. Brookings, 31-v-'27, H. C. S.; Madison, 9-vi-'29, H. C. S.

Lethocerus americanus (Leidy). Melham, 3-x-'28, H. C. S.

Belostoma flumineum Say. L. Hendricks, 23-vii-'22, H. C. S.; Brookings, 9-xi-'22, H. C. S.

A COMPARATIVE STUDY OF THE GERMICIDAL ACTIVITY OF CERTAIN COMPOUNDS¹

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With the introduction of many new disinfectant and antiseptic solutions in the last few years it has become increasingly important to study and to compare their germicidal properties. The usually accepted standard of comparison at present is the phenol coefficient. Phenol coefficient determinations are of value in demonstrating the potential usefulness of a disinfectant by indicating its relative germicidal power under specific conditions. There is, however, no serum or other natural protein material present (except that in the test culture) in the phenol coefficient test, although such substances would ordinarily be expected to markedly influence the germicidal efficiency of a material applied on the body.

The present study began with the determination of phenol coefficients of a number of compounds. From this point further experiments were carried out to ascertain the actual time required for each of the test germicidal materials to kill various types of organisms in the presence of blood plasma, and finally, studies on the bacteriostatic effects of the same materials were made.

In this study various solutions and mixtures of *sec*-amyltr cresol (pentacresol) and *o*-hydroxyphenylmercuric chloride (mercarboline) were compared with several widely used proprietary preparations.

Among these proprietary substances were included three mercurials (designated as mercurials A, B and C) and two phenol derivatives which will be referred to as phenolic compounds M and N.

TABLE 1. *Composition of sec amyltr cresol and o-hydroxyphenylmercuric chloride solutions tested*

Designation	Percentage						Pigment
	Alcohol	<i>sec</i> -amyltr cresol	<i>o</i> -hydroxyphenylmercuric chloride	Acetone	Soap	NaCl	
Tincture mercresin	50	0.1	0.1	10	—	—	+
Tincture mercarboline	50	—	0.1	10	—	0.9	—
<i>sec</i> -amyltr cresol soap sol.	—	1.0	—	—	2	—	—
Tincture pentacresol	50	0.1	—	10	—	—	—

¹ The work reported here was supported by a grant from the Upjohn Company.

PHENOL COEFFICIENT

The determination of phenol coefficients was according to the methods employed in the insecticide control laboratory of the Food and Drug Administration of the United States Department of Agriculture (1). Tests were run using *Eberthella typhosa* (Hopkins strain) at 20° C. and *Staphylococcus aureus* (F.D.A. No. 209) at 20° C. and at 37° C. The medication tubes were held at these temperatures in a water bath regulated so that the variation from the temperature specified was not more than $\pm 0.1^\circ$ C. Because of the highly bacteriostatic properties of the mercury compounds, the F.D.A. method of secondary subcultures was employed. This consisted of transferring four loopfuls of broth from the primary subcultures to secondary subculture tubes. The secondary transfers were made immediately after the primary subcultures were completed. When the mercurials were tested with *Staph. aureus*, secondary subcultures were found to be indispensable in the determination of the true phenol coefficient. When *Eb. typhosa* was the test organism, however, secondary subcultures were found to be unnecessary, for growth never occurred in the second tube unless it was also present in the primary subculture tube. Frequently the primary tube showed growth while the secondary tube remained sterile, indicating that too few *Eb. typhosa* organisms were carried over to the second tube to initiate growth. Table 2 presents a summary of the phenol coefficient determinations.

Examination of the results of the phenol coefficient determinations discloses several interesting facts. First, the 37° C. phenol coefficient (*Staph. aureus*) was in almost every case higher than that at 20° C. The effect of temperature was particularly marked in the case of the pentacresol (soap solution), mercurial C and mercresin. The phenol coefficient figures for these substances at 37° C. were more than double those at 20° C., indicating that the temperature coefficient for those compounds was greater than that for phenol.

The results presented in table 2 show a distinctly selective action by certain of the compounds. The phenol coefficient of *sec*-amyltricresol tincture (pentacresol) against *Staph. aureus*, was twice that obtained with *Eb. typhosa*. With *Eb. typhosa* as the test organism, mercarbolidide gave a coefficient about six times as high as that obtained when *Staph. aureus* (37° C.) was employed. The selective action of the *sec*-amyltricresol soap solution was even more marked. At 20° C. it gave a phenol coefficient of 1.8 for *Eb. typhosa*, whereas, for *Staph. aureus* it was 50, or about 28 times as great.

The influence of the solvent on germicidal activity and specificity is indicated by comparison of the soap solution with the tincture of *sec*-amyltricresol. At 20° C. the tincture was found to be approximately 17 times as effective against *Eb. typhosa*, whereas, against *Staph. aureus* (37° C.) the soap solution of *sec*-amyltricresol was more than twice as effective as the tincture. Dunn (2) in an independent study with *sec*-amyltricresol reports this compound to be particularly effective against gram positive organisms.

GERMICIDAL TESTS

In addition to comparisons of the various antiseptic substances with respect to their phenol coefficients, studies were made to determine the actual time required by these materials to kill five different species of

TABLE 2. *Phenol coefficients of various compounds (F.D.A. method)*

Solutions tested	Phenol coefficient					
	Eb. typhosa 20° C.		Staph. aureus 20° C.		Staph. aureus 37° C.	
	Market solution	Active constituent	Market solution	Active constituent	Market solution	Active constituent
Mercresin	0.14	140	0.29	290
Tincture mercarboline	0.22	220	0.038	38
Tincture pentacresol	0.03	30	0.06	60
Sec-amyltri-cresol (in soap)	0.018	1.8	0.5	50	1.3	130
Phenolic compound M	5.1(0.1) *	2.6(0.05)	
Phenolic compound N	0.06	60	0.06	60	0.06	60
Mercurial A	0.025	1.25	0.035	1.75
Mercurial B	0.033	33	0.025	25	0.035	35
Mercurial C	0.025	50	0.06	12

* Figures in parenthesis represent the phenol coefficient if adjusted to the dilution recommended for use.

bacteria. Tests were usually carried out in the presence of blood plasma in order to approach more nearly conditions under which the disinfectants would normally be used. Serum has been shown to inhibit or decrease the germicidal activity of many substances, especially the compounds containing mercury. The organisms used were *Staph. aureus*, *Streptococcus viridans*, *Eb. typhosa*, an aerobic spore former, *Bacillus metiens*², and an anaerobe, *Clostridium welchii*. All experiments were conducted at room temperature (about 25° C.).

The general procedure followed was to arrange a series of ten medication tubes. Into each tube was pipetted 2.0 cc. of dilute plasma or distilled water and 0.5 cc. of a 24-hour broth culture of the test organism. Finally, at intervals of 30 seconds, 2.0 cc. of disinfectant were added to each tube. After an interval of 30 seconds from the addition to the last tube, a loopful of the mixture was transferred from the first tube to a tube of broth. Subcultures were made from each tube in turn at 30 second intervals until 25 or 30 minutes had elapsed. In this way cultures were made from each medication tube at five minute intervals. All subcultures from mercurials were again subcultured by transferring four loopfuls to another tube of broth. Cultures were incubated at 37° C. for 48 hours before results were read. An occasional deviation from this procedure was the mixing of the disinfectant and plasma followed by the addition of the

² *B. metiens* is a gram positive spore forming aerobe which Levine and associates have found serviceable for studies on the germicidal properties of alkalies and chlorine compounds.

culture at 30-second intervals. The treatment varied, in some details, with the organism used. All cultures and subcultures of *Staph. aureus*, *Strep. viridans* and *Eb. typhosa* were made in standard F.D.A. broth.

When *B. metiens* was used, dried spores were weighed and added to a tube of distilled water to make a suspension of about 5,000,000 spores per cc. immediately before use in order to avoid errors which might result from germination of spores. This suspension was added to the medication tubes in amounts of 0.5 cc. Since the spores proved to be very resistant to the disinfectants, the intervals between subcultures were much longer than when *Staph. aureus* was used and extended over a much longer period of time.

Cultures of *Cl. welchii* were grown in egg-meat medium. This medium was also used for subcultures from the medication tubes, but it was found to be difficult to read results, especially if growth was slight. An improved medium in which it was easy to detect growth was prepared by adding to each tube of egg-meat medium 6 to 10 cc. of sterile milk. Growth of the organism was indicated by the characteristic stormy fermentation of the milk.

Results of these experiments are presented in table 3. It is worthy of note that in the tests with plasma, as in the phenol coefficient determinations, *sec*-amyltricrosol is more effective against *Staph. aureus* than is the mercarbolid. The soap solution of *sec*-amyltricrosol proved to be more effective against the spores of *B. metiens* than pentacresol (tincture *sec*-amyltricrosol). The mercarbolid was uniformly less effective against the gram positive cocci than were the pentacresol preparations.

When *Sertp. viridans* was the test organism, mer cresin was the only substance which killed that organism within five minutes in every trial when plasma was present. The culture survived in the phenol, mercurial A, mercurial B, mercurial C, and mercarbolid test solutions for 30 minutes but was killed in 16 hours, which was the next interval of time observed. Mercurial B failed to kill *Strep. viridans* even after 16 hours exposure. The selective action of the different mercurials for specific organisms is particularly worthy of note. Thus mercurial B which was as effective against *Staph. aureus* as mercurials A and C (see table 2) was apparently innocuous to *Strep. viridans*.

The test substances were found to be comparatively ineffective against the spores of *B. metiens* and *Cl. welchii*. Phenol, the cresol compound, mercurial B, pentacresol and mercarbolid failed to destroy the spores of *B. metiens* in 21 hours. Mer cresin and the *sec*-amyltricrosol (soap solution) were effective in 21 hours, while the mercurials A and C killed in five hours. It is interesting that three of the four substances effective against this organism were mercury compounds. With respect to *Cl. welchii*, 5 per cent phenol killed the organism in 21 hours. The mercurials A and C required 76 hours and 120 hours, respectively. The organism survived for 120 hours in all of the other test substances. The results presented in table 3 indicate that phenol is less effective against *B. metiens* (spores) than the mercurials A and C, but more effective against *Cl. welchii* than are those compounds.

BACTERIOSTATIC EFFECTS

A series of tests was carried out to determine the highest dilution of each disinfectant which would prevent growth of the test organisms dur-

ing a given period of observation. *Staph. aureus* and *B. metiens* were used as test organisms.

The technique employed was briefly as follows: In one series (broth) 1 cc. of a standard F.D.A. broth culture of *Staph. aureus* was added to 99 cc. of F.D.A. broth, 9 cc. of this bacterial suspension was added to test tubes containing 1 cc. of various concentrations of test materials, and the tubes placed in the 37° C. incubator. Another series (plasma) was carried out as above, except that the bacterial suspension consisted of 1 cc. of culture in 99 cc. of F.D.A. broth containing 10 per cent plasma.

Results of these experiments are summarized in table 4. It is apparent that the concentrations of these substances necessary to inhibit the test organisms were considerably greater in the presence of 10 per cent plasma than in broth.

All of the compounds containing mercury inhibited growth in higher dilution than the phenolic compounds. Mercresin was effective in higher dilution than the other mercurials tested.

The procedure with *B. metiens* was similar to that described for *Staph. aureus* except that a suspension of spores of *B. metiens* in broth was used as the inoculum. Tests were run both in F.D.A. broth and in 10 per cent plasma broth.

Results of these tests are presented in table 4. Concentrations necessary to inhibit these organisms were somewhat greater than those required when *Staph. aureus* was used and likewise greater in the presence of serum than in plain broth.

The compounds containing mercury inhibited growth in higher dilution than the non-mercurials, but no one of them was particularly effective against *B. metiens*.

SUMMARY

The phenol coefficient of the tincture of *sec*-amyltr cresol was found to be about 17 times that of the same compound in 2 per cent soap solution, when *Eb. typhosa* was employed as the test organism. On the other hand, when *Staph. aureus* (37° C.) was employed, the soap solution of *sec*-amyltr cresol was more than twice as effective as the tincture.

These *sec*-amyltr cresol, in 2 per cent soap solution, was found to possess definite selective action against *Staph. aureus*. At 20° C. it gave a phenol coefficient of 1.8 with *Eb. typhosa*, whereas, for *Staph. aureus* it was 50, or about 28 times as great.

In the presence of blood plasma mercresin was found to be more effective against *Strep. viridans* than any of the other compounds tested. The pentacresol preparations were uniformly more effective against the gram positive cocci than mercarbolide, when plasma was present.

Against the spore-formers, *B. metiens* and *Cl. welchii*, none of the substances were particularly effective, in the concentrations employed.

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TABLE 3. *Germicidal tests*

Test organism	Strep. viridans (.5 cc.)	Staph. aureus (.5 cc.)	Eb. typhosa (.5 cc.)	B. metiens (.5 cc.)	Cl. welchii (.5 cc.)
Medium of exposure	4 cc. 10 percent plasma 1 cc. disinfectant	2 cc. 20 per cent plasma 2 cc. disinfectant	2 cc. 20 per cent plasma 2 cc. disinfectant	5 cc. disinfectant	5 cc. disinfectant
Substances tested**	Killing time				
Mercurin	5 min.	5 min.	5 min.	21 hrs.	d
Mercurbolide	a	20 min.	5 min.	c	d
Pentracresol	15 min.	5 min.	5 min.	c	d
Sec. amylicresol*	10 min.	5 min.	5 min.	21 hrs.	d
Mercurial A	a	5 min.	5 min.	5 hrs.	76 hrs.
Mercurial B	b	25 min.	5 min.	c	d
Mercurial C	a	5 min.	5 min.	5 hrs.	120 hrs.
Phenolic compound N	10 min.	5 min.	5 min.	c	d
Phenol (5%)	a	5 min.	5 min.	c	21 hrs.

* 1 per cent sec-amylicresol in 2 per cent soap solution.

** Initial concentration of test materials is that offered to the public. Concentration in tests may be computed from composition of medium employed for exposure.

a. Not killed in 30 minutes but dead on examination after exposure of 16 hours.

b. Not killed after exposure for 16 hours.

c. Not killed after exposure for 21 hours.

d. Not killed after exposure for 120 hours.

TABLE 4. *Influence of plasma on bacteriostasis*

Tested substances	Staph. aureus			Bacillus metiens		
	Dilutions*			Dilutions*		
	Preventing growth		Permitting growth	Preventing growth		Permitting growth
	Broth	Plasma	Broth	Broth	Plasma	Plasma
Mercresin	1-1600	1-400	1-3200	1-500	1-250	1-500
Mercarboline				1-500	1-250	1-500
Pentacresol				1-20	1-10	1-20
Sec-amyltricresol	1-300	1-150	1-400	1-320	1-160	1-320
Mercurial A	1-400	1-400	1-1600	1-500	1-250	1-1000
Mercurial B	1-1600	1-200	1-3200	1-1000	1-250	1-500
Mercurial C	1-800	1-400	1-1600	1-500	1-250	1-500
Phenolic compound M	1-20	1-20	1-30			
Phenolic compound N	1-20	1-10	1-30	1-40	1-10	1-20
Phenol	1-400	1-200	1-600	1-200	1-200	1-400

* Concentration of compounds as offered to the public is taken as unity, except for phenolic compound M which is based on the dilution recommended for use (2 per cent), and phenol which is based on the pure substance.

METHOD OF PENETRATION, FORMATION OF STYLET SHEATHS AND SOURCE OF FOOD SUPPLY OF APHIDS¹

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The method of penetration of plant tissues and the particular tissues sought as a source of food supply by aphids are a part of the investigations connected with a study of insects as vectors of yellow dwarf, a virus disease of the cultivated onion. Since aphids are involved in the transmission of a greater number of virus diseases of plants than any other group of insects, a correct understanding of their feeding habits and the resulting effect on the plant tissues is of considerable importance. This work is a continuation of studies dealing with the intercellular abnormalities (Tate, 1935) associated with onion yellow dwarf, and has been conducted under the supervision of Dr. J. N. Martin of the Botany Department of Iowa State College.

Experimental evidence (Drake, Tate and Harris, 1932 and 1933) has convincingly demonstrated that in Iowa the virus of onion yellow dwarf is disseminated by various species of aphids, none of which normally feed on the onion plant. Moreover, when aphids are limited to such plants for their food supply most of them die within a period of 3 to 7 days. Somewhat similar virus-insect relationships have been established in connection with certain other diseases such as bean mosaic (Zaumeyer and Kearns, 1936) and sugar cane mosaic (Brandes, 1920). It, therefore, became of interest to determine if the different species of aphids, when confined upon plants which do not normally serve as hosts, feed in a manner similar to that on preferred hosts. In conjunction with this work, studies were also made with various species of aphids which had not formerly received attention from this point of view on their normal host plant for the purpose of obtaining further information on the feeding methods of aphids in general.

REVIEW OF LITERATURE

The effect of feeding punctures of Hemipterous insects and the particular region of the plant tissues penetrated has received the attention of a number of investigators and some of those most closely related to the present problem will be briefly reviewed.

Horsfall (1923) conducted experiments on the effect of feeding punctures of aphids on plant tissue in which four species were considered—namely, *Aphis rumicis* L., *Myzus persicae* Sulz., *Macrosiphum rudbeckiae* (Fitch), and *M. ambrosiae* (Thomas). He concluded that, as a rule, the

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path of the setae is intercellular and that the objective of the stylets is always a vascular bundle.

Davidson (1923) published the results of experiments in which he demonstrated that the piercing organ of aphids passed intercellularly through the cortex to the vascular bundle and only occasionally passed through individual cells.

Smith (1926) made a comparative study of the feeding methods of a number of species of Hemipterous insects, including three species of aphids. The result of his studies showed that in general the stylet track was intercellular with the phloem of the vascular bundle as the main objective.

MATERIAL AND METHODS

The following species of aphids and the host plant tissue upon which they were feeding at the time of being killed were used in these investigations: *Aphis rumicis* L. on nasturtium (*Tropaeolum majus* L.), dock (*Rumex crispus* L.), white sweet clover (*Melilotus alba* Desr.), snowball (*Symphoricarpos* sp.), and onion (*Allium cepae* L.); *Myzus persicae* Sulz. on onion (*A. cepae* L.); *Macrosiphum ambrosiae* (Thomas) on ragweed (*Ambrosia trifida* L.); *M. gei* Koch on milkweed (*Asclepias* sp.); *M. erigeronensis* (Thomas) on *Solidago canadensis* L.; *M. rudbeckiae* (Fitch) on *Silphium perfoliatum* L.; *Periphyllus negundinis* (Thomas) on box elder (*Acer negundo* L.); *Capitophorus ribis* L. on currant (*Ribes sativum* Syme) and on gooseberry (*Ribes* sp.); *Aphis pomi* DeGeer on apple (*Pyrus malus* L.); *Drepanaphis acerifoliae* (Thomas) on maple (*Acer saccharinum* L.); *Anoecia querci* (Fitch) on dogwood (*Cornus* sp.); *Prociphilus fraxinifolii* (Riley) on ash (*Fraxinus americana* L.); *Hysterneurosetariae* (Thomas) on plum (*Prunus domestica* L.); *Myzocallis puncta* (Monell) on oak (*Quercus* sp.); and *Aphis spiraeicola* Patch on *Spirea prunifolia* Sieb. & Zucc.

The object of the investigations was to determine the course of the setae through the plant tissues and the particular region sought as a primary source of food supply. An effort was made to select a wide range of aphid species from various types of plants. None of the species studied induces true gall formation but in some cases heavily infested plants exhibited marked macroscopical abnormalities such as curling and rolling of the leaves and the formation of hypertrophied areas. In obtaining material for study only portions of the plant heavily infested with aphids were selected in order to increase the probability of securing sections which showed either the setae or the setal sheaths in their entirety. Most of the material was collected at odd times while engaged in field experiments on insect transmission of onion yellow dwarf.

Considerable difficulty was experienced at first in the killing and the fixation of material because it was highly desirable to obtain the setae of the aphids in a natural feeding position within the plant tissues. The method finally selected as being most satisfactory was to transfer the pieces of plant tissues infested with aphids to a large "cyanid bottle" and as soon as the aphids had become perfectly quiet the tissue was dropped into the fixing agent.

During the early part of the work four different killing and fixing solutions were tried—namely, Bouin's, chromacetic, 95 per cent alcohol (hot), and alcohol-formalin acetic acid. The chromacetic acid solution was perhaps the most satisfactory since it caused little or no plasmolysis

of the plant tissues. Although the alcohol-formalin acetic acid solution resulted in some shrinkage it was generally used in fixing material in the field because of its convenience in handling. In carrying the tissue through the succeeding solutions many of the aphids which remained attached to the plant tissues by the setae at the time of killing and fixing were lost regardless of the most careful manipulation.

The tissues were sectioned 8-10 microns in thickness and then fixed on the slide and stained. A number of different stains were tried among which were included safranin with fast green, safranin with haemalum and safranin with gentian violet. In general the safranin and gentian violet combination gave the most satisfactory results.

In obtaining sections suitable for making photomicrographs both from the standpoint of plant tissue and aphid mouthparts it was necessary to prepare large numbers of slides. Little difficulty was experienced in most instances in obtaining sections of plant tissue in excellent condition or in locating either the aphid setae or stylet sheath in feeding position. The problem, however, of locating satisfactory combination of both plant material and insect stylets often proved to be very difficult. Occasionally it was necessary to select slides for photomicrographs in which the plant material was rather poorly stained and somewhat disorganized in order to obtain sections showing the entire course of stylets *in situ*.

THE SETAL PATH AND SOURCE OF FOOD SUPPLY

A study of 18 species of aphids *in situ* upon the leaf or stem of the host shows that in general the main objective of the setae is the vascular bundle. Although in the majority of species studied the setal path was intercellular as a rule a number of species followed a direct course through the cells and a few represented an intermediate group. Based on the type of stylet track the species of aphids studied form three main groups, namely:

1. Setal path intercellular to the vascular bundle.
2. Setal path either inter- or intracellular to the vascular bundle.
3. Direct path to the vascular bundle, regardless of cellular structure.

In this connection it is of interest to note the work of Busgen (1891), who observed three types of stylet tracks, namely: (1) Intercellularly to the phloem; (2) intracellularly through the parenchyma (no phloem objective); and (3) intracellularly to the phloem. It may be noted that his observations differ from those of the writer in that all of the species included in the present study appeared to penetrate the vascular bundle, whereas according to Busgen's observations the vascular bundle was not always the primary objective. In some cases (Plate I, fig. 4) setae and in other cases stylet sheaths (Plate IV, fig. 1) were observed terminating in the parenchyma, but it was concluded that in such cases either the penetration process had not been completed or the setae had been partially withdrawn at the time of killing. This may account for the difference between the findings of Busgen and the writer.

Although it would be impossible to place species invariably in one particular category each of those studied falls rather definitely into one of the three groups cited above. In the first group (i. e., setal path intercellular to the vascular bundle) belongs *A. rumicis* (Plate I, figs. 2, 3, 4, 5 and

6, Plate III, figs. 1, 2, 3 and 4), the setae of which were found almost invariably to follow an intercellular path in the four different host plants studied. In a few instances, however, *A. rumicis* was found to penetrate cells (Plate I, fig. 1). Although *M. persicae* was not studied on any of its normal hosts, sections of infested onion tissue show the setal path to be almost entirely intercellular (Plate II, a and b). These findings are in accord with those of Horsfall (1923), Davidson (1923), and Smith (1936).

In the second group (i. e., setal path either inter- or intracellular to vascular bundle) may be included the majority of the species studied but in most cases intercellular penetration was the more common method. These species are, namely, *M. rudbeckiae* (Plate VI, fig. 4), *M. gei* (Plate IV, fig. 1), *M. erigeronensis* (Plate IV, fig. 2), *M. ambrosiae* (Plate IV, fig. 3), *A. querci*, *D. acerifoliae* (Plate VII, fig. 2), *C. ribis* (Plate VI, fig. 2), *A. pomi*, *M. puncta* (Plate VIII, fig. 3), and *A. spiraeicola* (Plate V, fig. 2).

In the third category (i. e., direct path to the vascular bundle) *H. setariae* (Plate V, fig. 3), *P. fraxinifolii* (Plate VI, fig. 3), and *P. negundinis* (Plate VI, fig. 1) are good examples insofar as the material examined in the present studies is concerned. Stylet sheaths were very numerous in tissues of *Fraxinus americana* heavily infested with *P. fraxinifolii* and in all instances observed the setal path was direct through the parenchyma to the vascular bundle. In the case of *H. setariae* on *Prunus domestica* and *P. negundinis* on *Acer negundo* the stylet sheaths were much less numerous and, consequently, not so many sheaths were available for study.

It is of interest to note that numerous sections of onion plants showing stylet sheaths of both *A. rumicis* and *M. persicae* demonstrate that when these insects feed on plants not normally attacked they feed in a manner similar to that on their natural host plants.

THE STYLET SHEATH

During the penetrating and the feeding process of aphids the piercing organ, which is composed of the mandibular and maxillary stylets, is surrounded by saliva injected by the insect and by plant juices from the injured tissues. As a result of the action of the saliva, a precipitation or coagulation of substances occurs thus forming around the setae a thin walled tube referred to as the stylet sheath. When the setae are withdrawn the stylet sheath is left in the plant tissues and when treated with safranin the sheath stains a distinct reddish yellow color. Because of this property the course taken by the setae may be followed and the point of puncture determined even though the setae had been withdrawn at the time of or during the process of killing the material.

Although certain properties of the setal sheath have been determined, its function, its chemical and physical composition and the chemical reactions which take place during its formation are still imperfectly understood. Davidson (1923) is of the opinion that the sheath is composed of substances produced by the action of saliva on the cell sap, probably callose and insoluble calcium pectate together with tannin. Horsfall (1923) obtained evidence to indicate that the stylet sheath is composed of calcium pectate probably deposited by the plant cells and a proteid material evidently injected by the insect.

It is quite apparent that there exists considerable variation among aphid species with respect to the number of stylet sheaths that can be demonstrated by means of the safranin stain reaction. From slides of

leaves of *Ribes sativum* heavily infested with the aphid *C. ribis* numerous sections were examined but only a small number of sheaths were observed, whereas in tissue of *Fraxinus americana* infested with the aphid *P. fraxinifolii* stylet sheaths were numerous. A similar condition existed among several other species studied. Such differences are probably due to variations in the amount of saliva injected by the insect, the flow of plant juices, and the chemical reactions which follow and the responses of the resulting sheaths to stains. Of numerous sections of leaves of *Crataegus* infested with *Rhopalosiphum prunifoliae* (Fitch) examined no evidence of sheath formation was observed. The experiments with this species are not considered as being sufficiently intensive to warrant the obvious conclusion that a stylet sheath is not formed when it is feeding on *Crataegus*, but the technique employed failed to demonstrate the presence of sheaths.

In the sections examined the sheaths varied greatly in outline, being quite uniform in some sections (Plate IV, fig. 1, and Plate VII, fig. 4) while in other sections the outline was inclined to be irregular (Plate VII, figs. 1 and 3) as a result of unequally distributed deposits along the path of the stylets. In the sheath formed by *M. ambrosia* on ragweed (*Ambrosia trifida*) (Plate IV, fig. 3) there is a fairly uniform central sheath surrounded by an irregular dark stained deposit which probably is a result of saliva diffusing out into the adjacent area during the process of penetration.

SOME HISTOLOGICAL ABNORMALITIES ASSOCIATED WITH APHID FEEDING

In many of the plants examined sections of leaves heavily infested with aphids showed areas of abnormal tissue resembling pseudo-vascular tissue. These abnormalities were noted in the following plants: apple (*P. malus*), ash (*F. americana*), box elder (*A. negundo*), *Crataegus* sp., dogwood (*Cornus* sp.), currant (*R. sativum*), gooseberry (*Ribes* sp.), plum (*P. domestica*), dock (*Rumex* sp.), and snowball (*Symphoricarpos* sp.). In Plate VIII, figs. 1 and 2, are shown photomicrographs of this type of pseudo-tissue from leaves of currant (*R. sativum*) and gooseberry (*Ribes* sp.). In all of the other plants mentioned above more or less similar conditions were observed. These abnormalities were associated in every case with leaves which had become curled, rolled or otherwise distorted as a result of heavy aphid infestations.

Various degrees of derangement of leaf tissue were noted within the same plant and in different plants ranging from the differentiation of a few abnormal cells to the formation of pseudo-vascular tissue. The extent of derangement or development of pseudo-vascular tissue probably depends upon such factors as: (1) severity of infestation, (2) duration of feeding activities, and (3) nature of plant and its reaction to the stimulus of the insect secretion.

SUMMARY

1. The results of studies concerned with the course of the setal path and the particular region of the plant sought as a source of food supply by aphids on 19 species of plants are presented. Fifteen species of aphids were involved in the studies.

2. Based on the course followed by the setae in penetration three types of setal paths were observed—namely, (1) intercellularly to the

vascular bundle; (2) either inter- or intracellularly to the vascular bundle; and (3) a direct path to the vascular bundle regardless of cells or intercellular spaces.

3. In all aphids studied the vascular bundle and particularly the phloem elements were found to be the objective of the setae and hence the principal source of food supply.

4. A study of sections of onion tissues infested with *Aphis rumicis* and *Myzus persicae*, neither of which normally feeds on this plant, showed the method of penetration by the setae and source of food supply to be similar to that on normal host plants.

5. With the technique employed the extent to which stylet sheaths could be demonstrated was found to vary considerably among different species of aphids and in different species of plants.

6. The plants which showed such symptoms as leaf curling, rolling or pseudo-leaf galls as a result of aphid attacks were found to exhibit marked histological abnormalities principally in the form of pseudo-vascular tissue.

7. A number of photomicrographs and drawings illustrating the points under consideration are presented.

PLATE I

Drawing showing the path followed by the setae in penetrating the plant tissue and the stylet sheaths formed by *A. rumicis*.

- Fig. 1. Transverse section of stem of nasturtium showing setae following an intracellular path to phloem. Note the irregular deposits along the setal path.
- Fig. 2. Transverse section of onion leaf showing stylet sheath following an intercellular path to the vascular bundle.
- Figs. 3 and 5. Transverse section of steam of nasturtium showing setae penetrating intercellularly to the phloem.
- Fig. 4. Transverse section of stem of nasturtium showing the setae following an intercellularly course and terminating in the cortical cells. It is quite likely that the penetrating process had not been completed at the time the material was killed.
- Fig. 6. Transverse section of stem of nasturtium showing stylet sheath following an intercellular path, one branch of which terminates in the intercellular spaces of parenchyma cells.

Reference Lettering

S=setae; T=tube or stylet sheath; I=intercellular space; Ph=phloem; X=Zylem; L=labium.

PLATE I

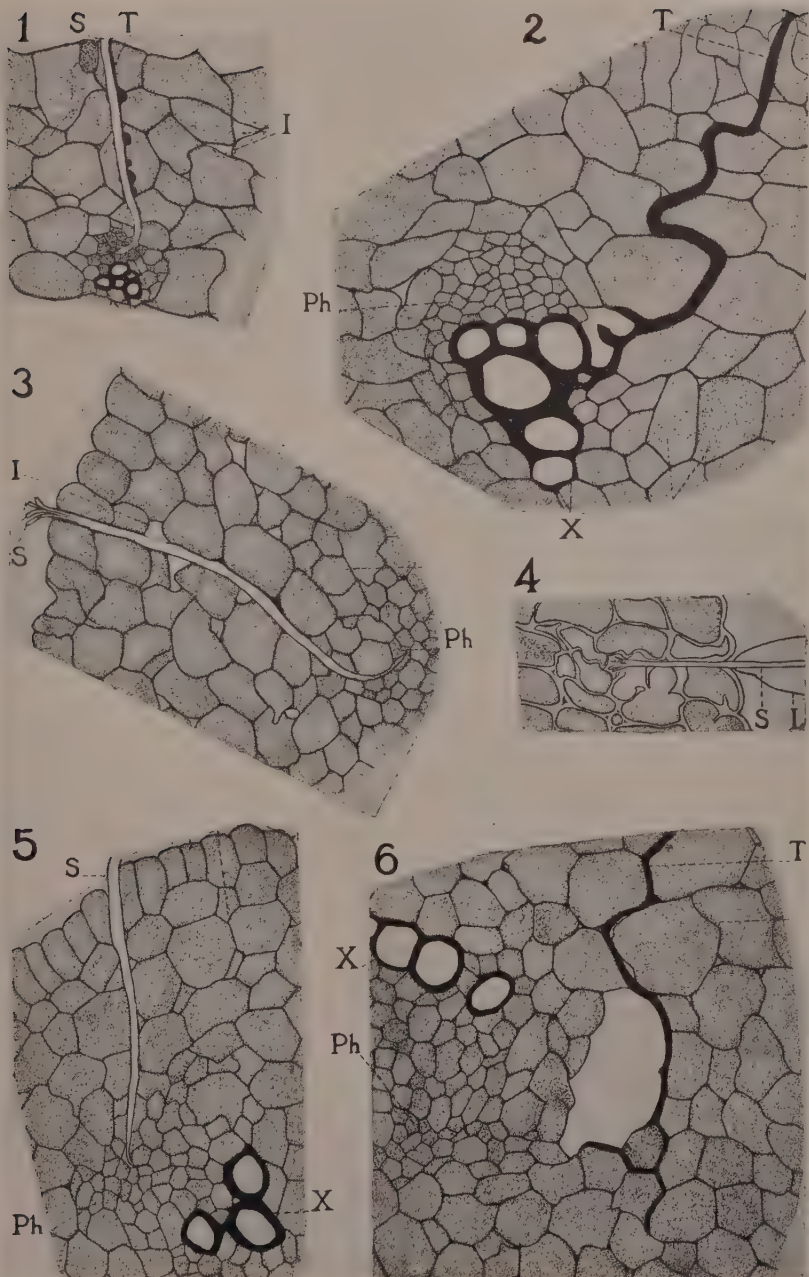


PLATE II

Drawings showing path-followed by the setae and stylet sheath formed by *Myzus persicae* in onion.

Fig. a. Transverse section of onion leaf showing setal path leading intercellularly to phloem. x 500.

Fig. b. Transverse section of onion leaf showing stylet sheath leading to phloem. x 500.

PLATE II

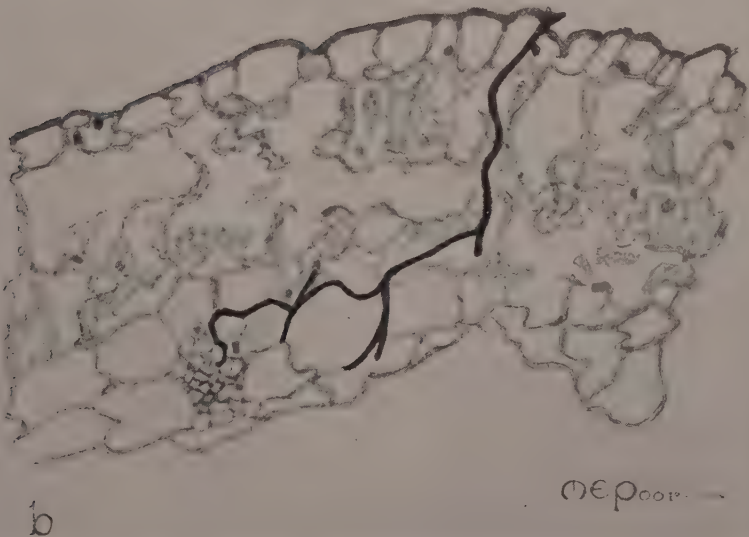
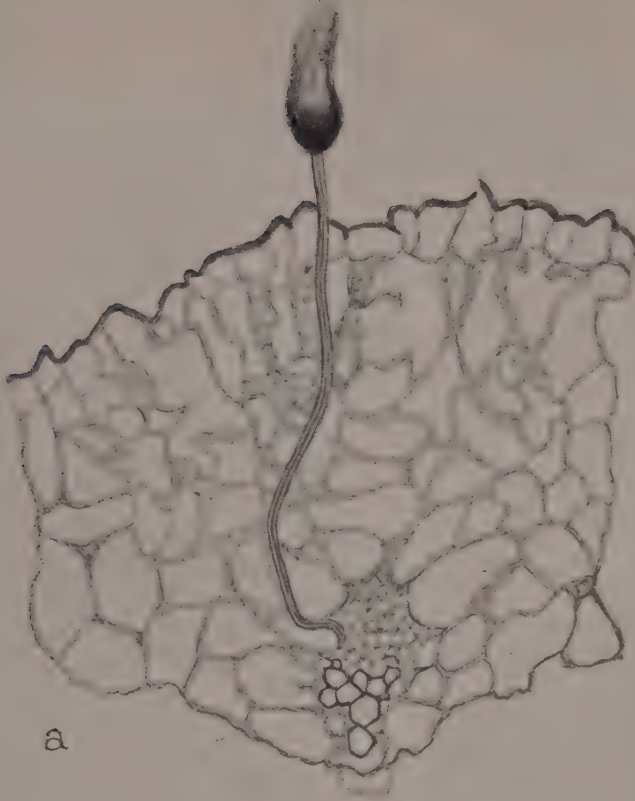


PLATE III

Photomicrographs showing path followed by setae and source of food supply of *A. runcidis* on nasturtium, *Rumex*, white clover, and snowball.

- Fig. 1. Cross section of stem of nasturtium showing intercellular course of the setae which terminates in the vascular bundle. x 265.
- Fig. 2. Cross section of stem of *Rumex* showing stylet sheath following an intercellular course to the phloem. It will be noted that a portion of the sheath is not present in this section. x 250.
- Fig. 3. Cross section of mid-vein of leaf of snowball showing stylet sheath leading to vascular bundle. x 250.
- Fig. 4. Cross section of stem of white clover showing stylet sheath following an intercellular path to the vascular bundle. x 250.

PLATE III

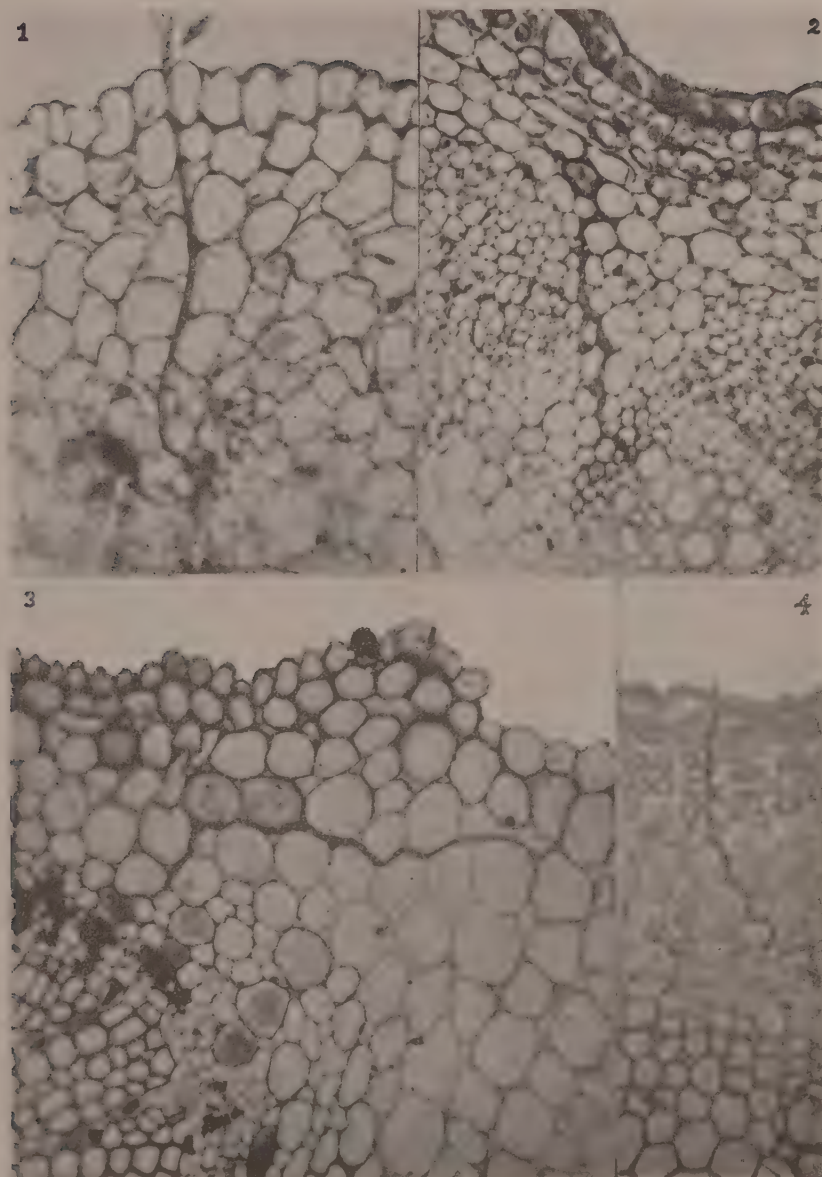


PLATE IV

- Fig. 1. Transverse section of milkweed leaf showing stylet sheath of *M. gei*. x 265.
- Fig. 2. Transverse section of stem of *Solidago* showing stylet sheath of *M. erigeronensis* leading to vascular bundle. x 220.
- Fig. 3. Transverse section of ragweed stem (*Ambrosia trifida*), showing the stylet sheath of *M. ambrosiae* leading to vascular bundle. x 265.

PLATE IV

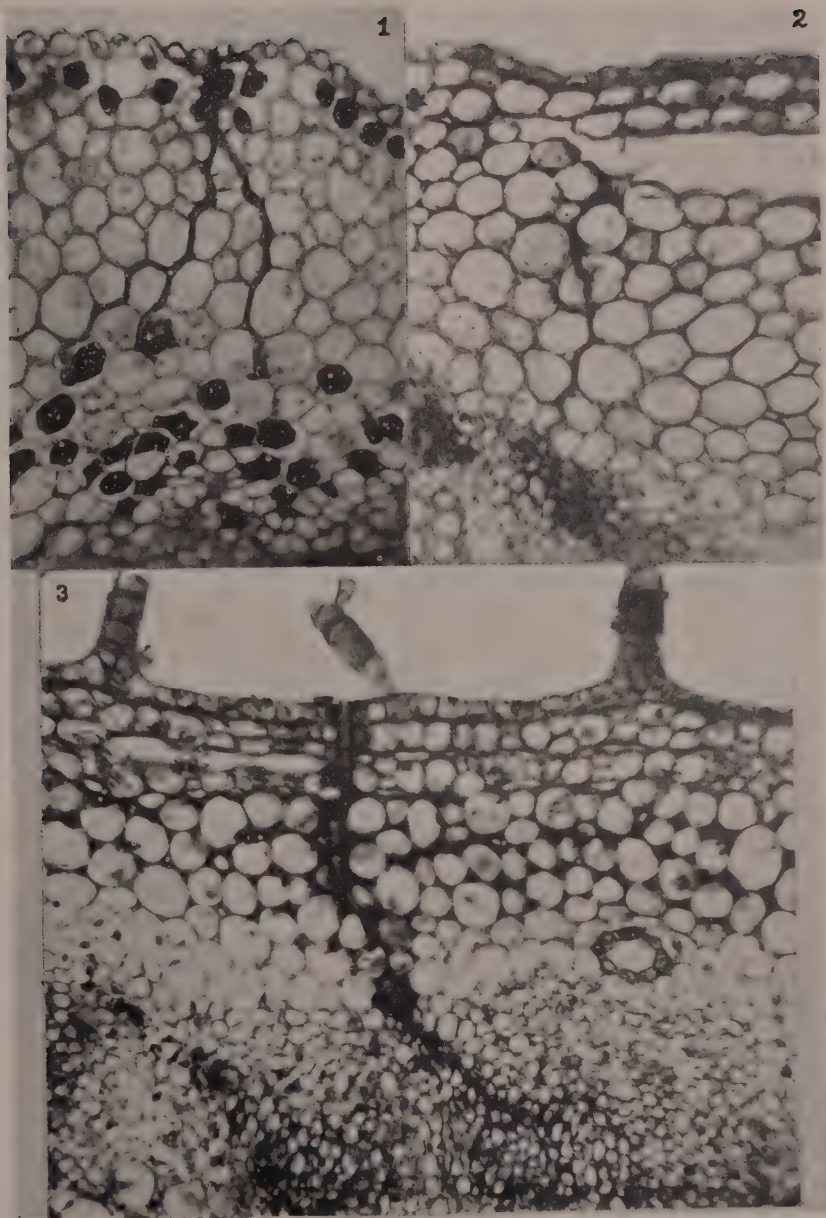


PLATE V

- Fig. 1. Cross section of leaf of dogwood, showing setae of *A. querci* penetrating vascular bundle. x 250.
- Fig. 2. Cross section of stem of *Spirac* showing stylet sheath of *A. spiraeicola* extending to the vascular bundle. x 265.
- Fig. 3. Longitudinal section of leaf of plum (*Prunus domestica*) showing a median longitudinal section of *H. setariae* with the setae in feeding position and penetrating the vascular elements.

PLATE V

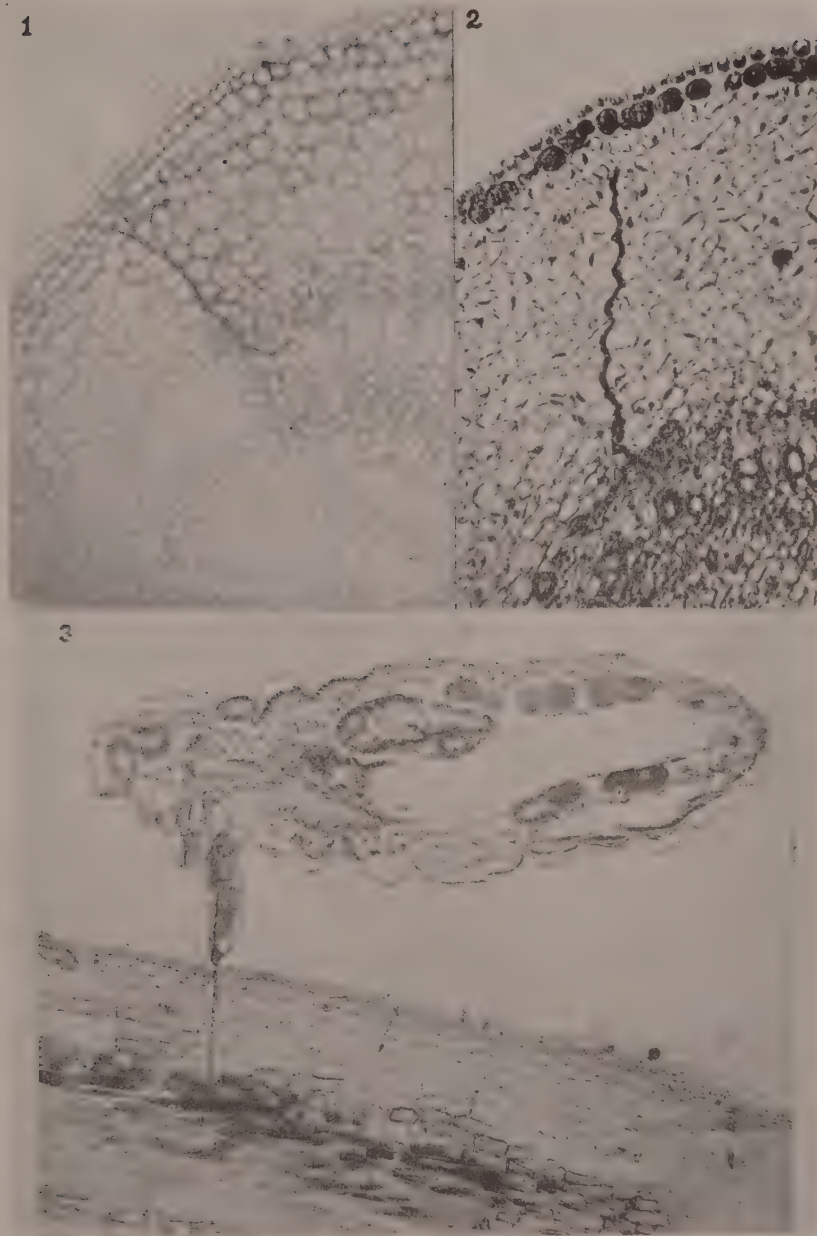


PLATE VI

- Fig. 1. Transverse section of mid-vein of box elder leaf (*Acer negundo*), showing setae of *P. negundinis* leading to the vascular bundle. x 250.
- Fig. 2. Transverse section of leaf of currant (*Ribes sativum*) showing stylet sheath of *C. ribis* following an intercellular path to the vascular bundle. x 235.
- Fig. 3. Longitudinal section of leaf of ash (*Fraxinus americana*) showing two stylet sheaths of *P. fraxinifolii* following a direct path to the vascular bundle. Note that the stylet sheath projects above epidermis. x 265.
- Fig. 4. Transverse section of stem of *Silphium perfoliatum* showing stylet sheath of *M. rudbeckiae* penetrating the phloem tissue. x 155.

PLATE VI

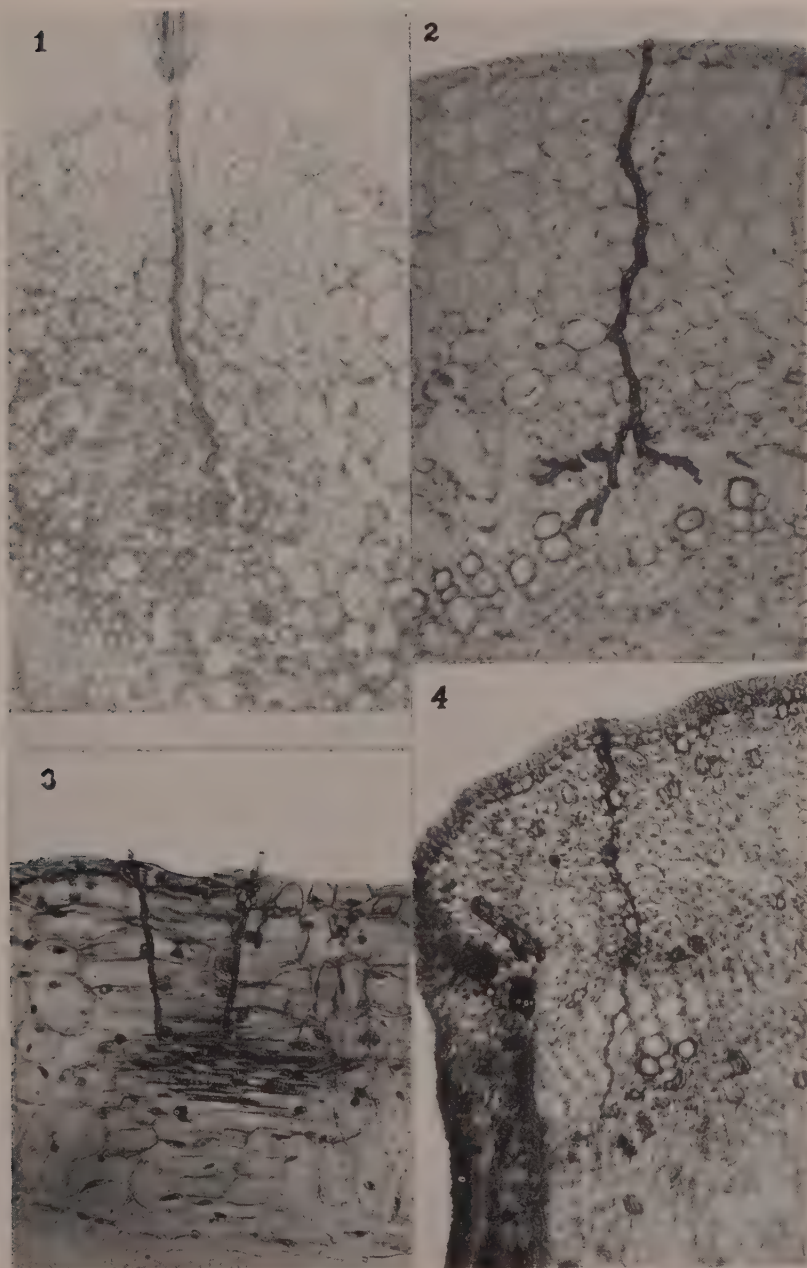


PLATE VII

- Fig. 1. Transverse section of mid-vein of apple leaf showing stylet sheath of *A. pomi* following an intercellular path to the phloem. x 265.
- Fig. 2. Transverse section of mid-vein of maple leaf showing stylet sheath of *D. acerfoliae* following an intercellular path to the phloem. x 265.
- Fig. 3. Transverse section of mid-vein of oak leaf showing stylet sheath of *M. puncta* following an intracellular path to the phloem. x 265.
- Fig. 4. Transverse section of leaf of gooseberry (*Ribes* sp.) showing stylet sheath of *C. ribis* penetrating phloem tissue. x 250.

PLATE VII

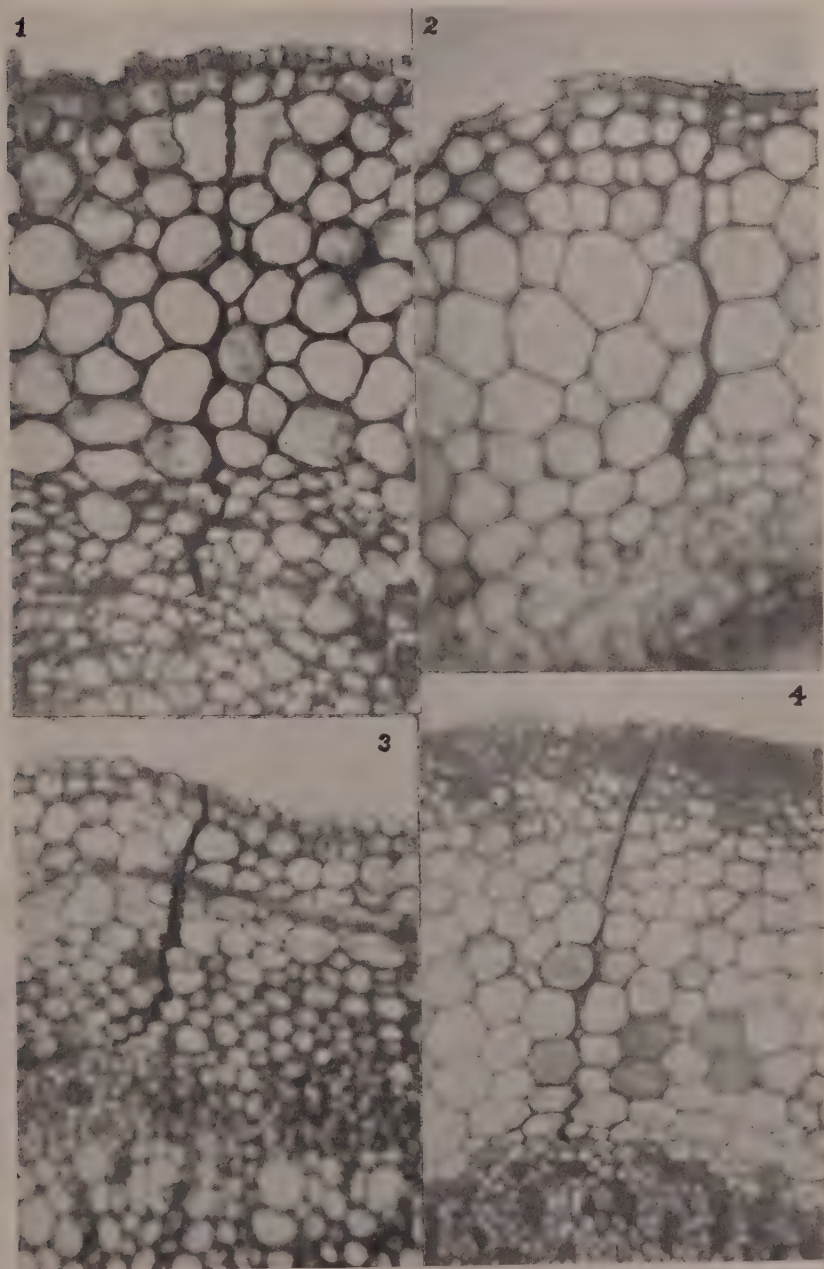
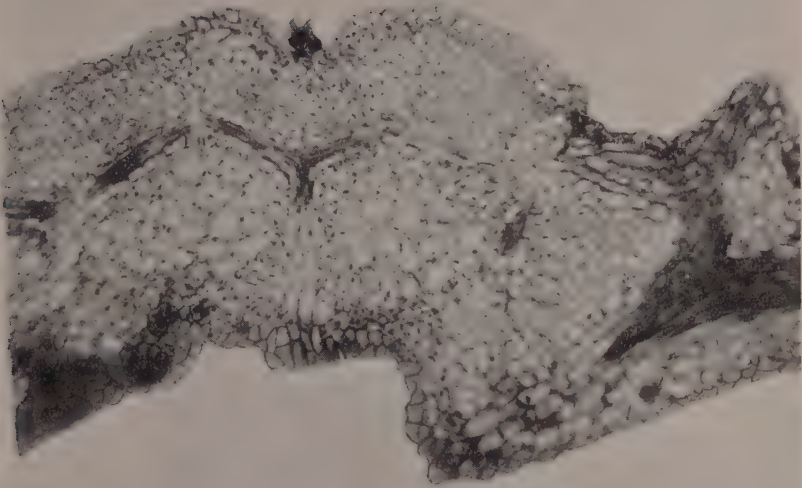


PLATE VIII

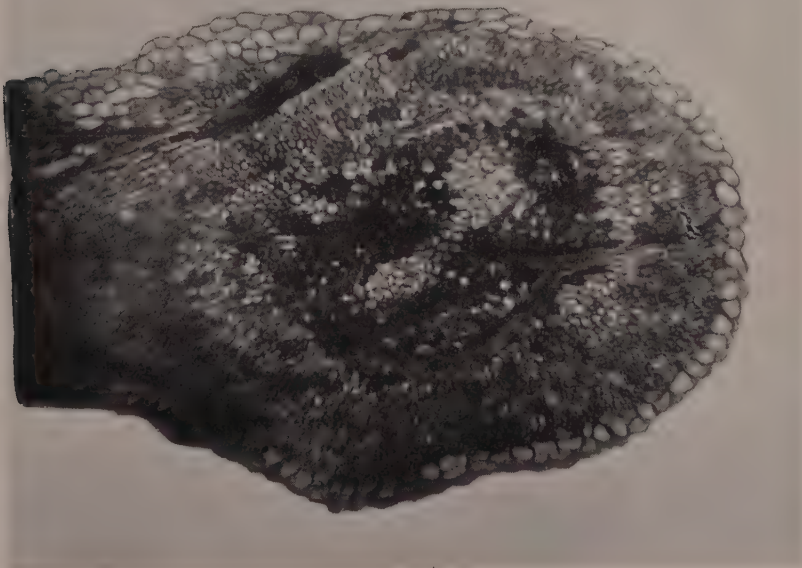
- Fig. 1. Showing development of pseudo-vascular tissue in leaf of gooseberry (*Ribes* sp.) infested with *C. ribis*.
- Fig. 2. Showing development of pseudo-vascular tissue in leaf of currant (*Ribes sativum*) infested with *C. ribis*.

PLATE VIII

1



2



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THE GENERA SPOROBOLOMYCES AND BULLERA FROM THE STANDPOINT OF DAIRY PRODUCTS¹

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In studies on the numbers of bacteria, yeasts and molds falling from the air in dairy plants, organisms were encountered which discharged spores from the colonies on inverted plates in such a manner that definite spots appeared on the covers exactly beneath the colonies. The organisms evidently belong to two genera, *Sporobolomyces* and *Bullera*. Although these genera have been studied by a number of investigators, little attention has been given them in the United States and, accordingly, an attempt was made to isolate cultures from various sources, particularly from dairy products. The cultures obtained were studied from the standpoint of their classification.

HISTORICAL

In 1923 Kluver and van Niel (3) isolated a number of red yeasts which were contaminants of yeast cultures from Japan. They found that when these yeasts were grown on inverted plates, mirror images of the colonies were produced on the covers of the plates by the shower of kidney-shaped cells discharged from the colonies. They noted that several investigators had previously studied these "spore-discharging" yeasts and had assigned to them various names, none of which in their opinion seemed justified. After studying the organisms in considerable detail, Kluver and van Niel proposed for them the generic name *Sporobolomyces*, the peculiar spore discharging characteristic distinguishing them from closely allied genera. Their six types were provisionally divided into three species, *Sporobolomyces salmonicolor*, *Sp. roseus* and *Sp. tenuis*, the division being made mainly on the basis of colony and physiological characteristics. These investigators gave a detailed description of the spore discharging mechanism and showed why the explanations of this phenomenon given by earlier investigators were erroneous.

In their study on the fungi of Manitoba, Bisby Buller and Dearness (1) encountered two species which they regarded as *Sporobolomyces*; these were *Sp. roseus* and a new species, *Sp. alba*, and were obtained from rusted wheat. The latter species differed from the other known members of the genus by the absence of any red coloring in the colonies; when grown on nutrient agar it was white first, then creamy white and finally sordid yellow.

Derx (2) found that *Sporobolomyces* were rather widely distributed in nature, occurring in the dust of air, on the seed and straw of grains, on growing plants, and on decaying leaves. They were frequently found liv-

¹ Journal Paper No. J391 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 119.

ing in the sugary plant exudates resulting from physical or parasitic injury. He studied 40 cultures which he divided into seven species of *Sporobolomyces* and two of *Bullera*, a new genus created for the organisms showing no trace of red color. In addition to the three species of *Sporobolomyces* described by Kluyver and van Niel (3) Derx recognized four new species, *Sp. salmoneus*, *Sp. gracilis*, *Sp. albo-rubescens*, and *Sp. odoratus*. The two species of the genus *Bullera* recognized were *Bullera alba* (*Sp. alba* of Bisby, Buller and Dearness) and *B. grandispora*. Derx found that variations, which are so persistent the variants may be considered as new species, sometimes occur suddenly, such as the loss of the ability to produce mucilage or the loss of color. He suggested that these variations are due to degeneration resulting from either the repeated transfer of only the projected cells as a means of conserving the purity of the culture, or the exhaustion of the projected cells in discharging secondary cells.

METHODS

The *Sporobolomyces* and *Bullera* cultures were isolated from acidulated (pH 3.5) malt agar or potato dextrose agar plates; many of these had been used in studies on the numbers of bacteria, yeasts and molds falling from the air (4) while the others had been inoculated with various materials which might harbor the organisms, such as butter having high yeast counts, various defective dairy products, and infusions of leaf mold or grass. The *Sporobolomyces* and *Bullera* cultures were easily identified by inverting inoculated plates over sterile malt agar plates and then picking the colonies which developed from the discharged spores that fell on the lower plates; in some instances all the yeast colonies on a plate were streaked on a sterile malt agar plate which was then inverted over a sterile agar plate while in others the original plates were inverted. Tree leaves and fruit peeling were examined by partially imbedding them in agar in a petri plate and inverting over sterile malt agar. All the cultures were purified by inoculating them on malt agar plates, inverting these over other plates and finally picking well isolated colonies that developed from discharged spores. An incubation temperature of about 21° C. was regularly used, except in the determination of the growth temperatures.

In measuring the organism, the vegetative cells were obtained from cultures grown on malt agar slopes for 24 hours while the spores were obtained by streaking the cultures on malt agar plates, incubating the inverted plates until visible quantities of discharged spores were deposited on the covers, and then suspending the spores in sterile water; the organisms were measured in aqueous suspension. Staining reactions were determined on malt agar slope cultures grown for 24 hours and also on older cultures. Attempts to demonstrate the formation of ascospores were made by gram staining old malt agar cultures and cells held on gypsum blocks for various periods.

Colony characteristics were observed with cultures grown on malt agar plates and slopes. Color production was characterized by comparing the colors produced on potato, and on slopes of malt agar and potato dextrose agar, with the color standard plates given by Ridgway (5).

Assimilation of carbon compounds was determined in broth containing 2.0 per cent yeast extract, 0.0016 per cent brom cresol purple and 2.0 per cent of a sugar or other carbon source, while assimilation of nitrogen compounds was determined in the medium employed by Kluyver and

van Niel (3); the inoculated tubes were observed after incubating 1, 2, 4, 8 and 14 days.

Growth temperatures were studied by streaking the cultures on malt agar plates, incubating at various temperatures and observing daily for evidence of growth.

SOURCES OF THE ORGANISMS

Of the 33 cultures of *Sporobolomyces* and *Bullera* isolated, 32 were obtained from malt agar plates that had been exposed to the air (3 being from plates exposed in a churn that had not been used for some time) and 1 by plating fermented cream on acidified malt agar. The attempts to obtain the organisms from green and decaying leaves, grass, peelings from spoiled and sound apples and oranges, various defective dairy products and numerous samples of butter having high yeast counts were unsuccessful.

IDENTITY OF THE ORGANISMS

On the basis of the morphological, cultural and biochemical characters, the 33 cultures were divided into four groups. Comparisons were then made with type cultures².

Group 1 included 12 cultures, of which 11 were isolated from the air (3 from plates exposed in a churn) and 1 from fermented cream; the organisms were identified as *Sp. salmonicolor*, although minor variations were noted among the cultures. Six of the cultures were the same color as the type culture of this species and 6 were more red. On solid media, 2 cultures were cartilaginous and powdery, like the type culture, and the other 10 were more slimy or mucilaginous. Six of the cultures gave abundant growth and 6 gave good growth on the medium containing alcohol as the carbon source but none of them developed an acid reaction as did the type culture. These differences are apparently not of enough importance to warrant the creation of new species.

Group 2 included 12 cultures, isolated from the air, which were identified as *Sp. roseus*. Minor differences were noted among the cultures and the characters of some of them suggested *Sp. tenuis* but, since the characters which have been used to distinguish between *Sp. roseus* and *Sp. tenuis* are apparently not stable, it appears that the organisms which have been designated *Sp. tenuis* should all be regarded as *Sp. roseus*.

Group 3 included 3 cultures, isolated from the air, which apparently belong to a new species. The organism differs from the other species of *Sporobolomyces* studied in several characters but principally in the maximum growth temperature (about 34° C.) and in its failure to use potassium nitrate as a source of nitrogen. The organisms resembled *Sp. roseus* more closely than any of the other species studied and the name *Sp. pararoseus* is suggested for it.

Group 4 included 6 cultures isolated from the air. The absence of any red color in the cultures and the size and shape of vegetative cells and discharged spores identified the organisms as *B. alba*.

DESCRIPTION OF THE SPECIES

The description of the new species, *Sp. pararoseus*, is presented and the description of *B. alba* is extended.

² The type cultures were obtained from Dr. A. J. Kluyver, Delft, Holland.

Sporobolomyces pararoseus sp. nov.

MORPHOLOGY

Form and size. The vegetative cells were oval, the majority measuring from 2.5 to 4.0 by 5.0 to 8.0 microns. The discharged spores were reniform and narrowed where attached to the sterigmata; the majority of the spores measured from 2.0 to 4.0 by 5.5 to 8.5 microns.

Arrangement. The vegetative cells were usually lying free or with one bud attached.

Staining reaction. Cells from young cultures were gram positive while in old cultures gram negative cells were also usually present.

Spore formation. Formation of ascospores could not be demonstrated. A fairly abundant discharge of the reniform spores occurred within 24 hours.

CULTURAL CHARACTERISTICS

Malt agar slope. Growth was smooth, shiny, mucilaginous and light jasper red at first, later becoming somewhat dull, slightly furrowed on the surface, butyrous in consistency, and light coral red.

Whey agar slope. Growth was scant.

Malt agar stab. Growth occurred principally at the surface.

Malt agar plate colony. The colonies were round, smooth, raised, entire and mucilaginous. Later the surface developed concentric furrows and became dull and slightly powdery.

Gelatin stab. In plain or yeast extract gelatin scant growth and slight liquefaction occurred at the surface.

Liquid media. Liquid media became turbid, then cleared, leaving a heavy sediment. A ring pellicle developed within 8 days.

Potato. Growth was abundant, spreading, shiny and jasper red.

Litmus milk. Litmus milk became alkaline followed by alkaline coagulation and partial digestion. After 56 days the upper one-half of the milk was digested leaving a clear, wine colored serum on top and an alkaline curd at the bottom.

Dunham's solution. Fair growth.

Uschinsky's solution. Scant growth.

BIOCHEMICAL FEATURES

Fermenting power. As sources of carbon, glucose, levulose, sucrose, maltose, raffinose and mannitol were assimilated readily, galactose, inulin, dextrin, starch, glycerol, salicin and alcohol less readily, while arabinose, rhamnose and lactose were not assimilated. With the easily assimilable compounds, acid was generally produced.

Nitrogen assimilation. As sources of nitrogen, ammonium sulfate, acetamide, asparagin and peptone were readily assimilated while potassium nitrate was not assimilated.

GROWTH CONDITIONS

Oxygen relationship. The organism grew well aerobically on various media.

Temperature relationship. The optimum growth temperature was about 27° C. and the maximum about 34° C.

Bullera alba

MORPHOLOGY

Form and size. The vegetative cells were oval, the majority measured from 3.5 to 3.9 by 5.7 to 7.6 microns. The discharged spores were citriform and apiculated, the majority measuring from 3.6 to 5.0 by 5.7 to 7.0 microns.

Arrangement. The vegetative cells were usually lying free or with one bud attached.

Staining reaction. Cells from young cultures were gram positive while in old cultures gram negative cells were commonly present.

Spore formation. Formation of ascospores could not be demonstrated. An abundant discharge of the citriform spores occurred within 24 hours.

CULTURAL CHARACTERISTICS

Malt agar slope. Growth was smooth, shiny, mucilaginous and creamy white. Some of the cultures became dull, cartilaginous and wrinkled within 4 days while with others this change did not occur until later. All the cultures changed to a capucine buff color within 14 days.

Whey agar slope. Growth was abundant.

Malt agar stab. Growth occurred mainly at the surface.

Malt agar plate colony. The young colonies were round, smooth, shiny, raised, entire, mucilaginous and creamy white. After 4 days the colonies of some of the cultures were dull, cartilaginous and wrinkled while the colonies of others remained mucilaginous for a considerable period.

Gelatin stab. In plain or yeast extract gelatin growth was poor but there was considerable liquefaction at the surface after 30 days.

Liquid media. Liquid media became turbid and then cleared, leaving a heavy sediment; a ring pellicle developed within 14 days.

Potato. Growth was abundant, smooth, shiny, mucilaginous and creamy white after 2 days and later became wrinkled, dull, cartilaginous and capucine buff.

Litmus milk. A slight acid reaction and a ring pellicle developed within 14 days and partial digestion was evident after 30 days.

Dunham's solution. Fair growth.

Uchinsky's solution. Scant growth.

BIOCHEMICAL FEATURES

Fermenting power. As sources of carbon, glucose, sucrose, maltose, lactose and raffinose were assimilated readily, arabinose, rhamnose, levulose, galactose, inulin, dextrin and mannitol less readily, while glycerol, starch, alcohol and salicin were not assimilated. An acid reaction generally developed with the readily assimilable compounds.

Nitrogen assimilation. As sources of nitrogen, peptone and asparagin were assimilated fairly well while ammonium sulfate, potassium nitrate and acetamide were only slightly assimilated.

GROWTH CONDITIONS

Oxygen relationship. The organism grew well aerobically on various media.

Temperature relationship. The optimum growth temperature was about 21° C. and the maximum about 27° C.

GENERAL ACTION OF THE ORGANISMS ON DAIRY PRODUCTS

The organisms grew well in litmus milk as indicated by heavy deposits of cells in the bottoms of the tubes; the *Sporobolomyces* cultures developed an alkaline reaction and slight digestion while *B. alba* produced a slight acid reaction and more extensive digestion; the changes occurred slowly. The isolation of only one culture of *Sporobolomyces* in numerous attempts with various dairy products suggests that the occurrence of these organisms in dairy products is unusual. The recovery of *Sp. salmonicolor* from the air in a churn that had stood idle for some time suggests the possibility of high yeast counts in butter resulting from a churn contaminated with *Sporobolomyces* organisms. Contamination of butter might result also from the organisms growing on the ceiling and walls of a butter plant and the discharged spores falling into the pasteurized cream or on the butter and the equipment.

Under ordinary conditions it appears that *Sporobolomyces* and *Bullera* organisms are unimportant in dairy products, either from the standpoint of numbers present or defects produced; the occasional occurrence of these organisms in dairy products is to be expected as a result of air contamination.

SUMMARY

Twenty-seven cultures of *Sporobolomyces* and six of *Bullera* were isolated and studied. Twenty-six of the *Sporobolomyces* were obtained from malt agar plates that had been exposed to the air (three being from plates exposed in a churn) and one from fermented cream. The six cultures of *Bullera* were obtained from malt agar plates that had been exposed to the air. Other materials examined such as leaves, grass, fruit peelings, various defective dairy products and butter having high yeast counts, failed to yield *Sporobolomyces* or *Bullera* organisms.

The cultures included *Sp. salmonicolor*, *Sp. roseus*, *B. alba*, and a new species for which the name *Sp. pararoseus* is suggested. It appears that the organisms which have been designated *Sp. tenuis* should be included in the species *Sp. roseus*.

The *Sporobolomyces* and *Bullera* organisms are apparently unimportant in dairy products, either from the standpoint of the numbers present or the production of defects.

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PLATE I

Illustration of a mirror image produced by a *Sporobolomyces* culture. The left malt agar plate was inoculated and then inverted over the right plate for two days. The culture used was *Sp. salmonicolor*.

PLATE I



Right

Left

ALCOHOL YIELDS FROM ACID SACCHARIFIED CEREALS

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The saccharification of starch may be accomplished by certain diastase producing molds, malt, or by means of acid hydrolysis. Malt has proven the most satisfactory saccharifying agent from the standpoint of high alcohol yields. Theoretically, on the basis of available sugars, acid hydrolysis should prove just as effective.

The purpose of this investigation was to determine optimum conditions for the acid hydrolysis of starch to fermentable sugars with subsequent experiments involving comparison of alcohol yields from acid hydrolyzed and malted grains.

INFLUENCE OF TIME, PRESSURE AND ACID CONCENTRATION UPON SACCHARIFICATION OF STARCH OF VARIOUS GRAINS

Each hydrolysis was carried out in a 300 cc. Erlenmeyer flask placed in a steam autoclave. Fifteen grams of grain were taken to 100 cc. of the HCl solution, the final volume after hydrolysis amounting to approximately 106 cc. In timing the period of hydrolysis, 10 minutes were allowed after the steam was turned on to permit the contents of the flasks to reach the temperature to be maintained in the autoclave. This allowance also included the time during which air was being driven from the autoclave.

Reducing sugars were determined and calculated as glucose although it was recognized that this was not an absolute index to the actual amount of fermentable carbohydrates as other reducing substances may have been present.

The following tables (1, 2, 3, 4) show the results obtained with the acid hydrolysis of wheat, corn, oats and barley, varying time, temperature and acid concentration. In each case HCl was the acid employed.

FERMENTATION OF ACID HYDROLYZED GRAINS

Having found the various times and pressures together with the corresponding acid concentrations giving the highest conversion of the starch into reducing sugars, fermentations were run in the different acid hydrolyzed media after adjusting the hydrolysate to a pH of 5 with NaOH. Alcohol determinations were run on 200 cc. samples of the fermented mash, distilling 100 cc. in volumetric flasks. Analyses were made by taking the specific gravities and converting these readings to the corresponding alcohol concentrations.

While approximately the same optimum reducing sugar concentrations were obtained using high acid concentrations and low temperatures as with lower acid concentrations and higher temperatures, the alcohol

¹ Journal Paper No. J434 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 347.

TABLE 1. *Influence of time, pressure and acid concentration upon the acid saccharification of wheat*

(Percentage dry grain in acid solution = 12.9)

N. of HCl soln.	Yield glucose (basis dry grain)	N. of HCl soln.	Yield glucose (basis dry grain)
Cooked $\frac{1}{2}$ hr. Atm. pressure		(Cooked 1 hr. 10 lbs. pressure)	
0.0	0.0	2.2
0.15	14.9	0.1	40.1
0.30	27.8	0.2	65.0
0.45	46.1	0.3	70.5
0.60	58.4	0.4	71.3
0.75	61.0	0.6	71.3
0.90	63.6	0.7	69.0
1.25	69.8	0.8	71.2
1.50	72.0	0.9	70.5
2.00	72.0	1.0	69.8
(Cooked 1 hr. Atm. pressure)		(Cooked 1 hr. 20 lbs. pressure)	
0.0	0.0	1.4
0.25	41.1	0.25	61.6
0.50	63.5	0.50	72.9
0.75	74.3	0.75	72.9
1.00	74.6	1.0	73.2
1.25	74.0	1.25	75.8
2.75	70.5	1.50	73.2
		2.00	69.7
		2.75	69.7
(Cooked 2 hrs. Atm. pressure)			
0.0	1.4		
0.1	16.0		
0.2	36.7		
0.3	56.8		
0.4	63.8		
0.5	70.7		
0.6	72.2		
0.7	71.6		
0.8	71.5		
0.9	75.1		
1.0	71.1		

yields did not coincide in the same manner. In general, better alcohol yields were obtained using the lower acid concentrations and higher temperatures over a longer period of time. As may be seen from the following table (5), the best yields were obtained from oats, wheat and barley, using an acid concentration of 0.15 N. over a period of 2 hours at 25 lbs. pressure, or 0.1 N. HCl over a period of 3 hours at 25 lbs. pressure.

The best yields of alcohol from corn were obtained using an acid concentration of 0.1 N., pressure, 25 lbs., and time, 2 or 3 hours; or an acid concentration of 0.105 N., pressure, 25 lbs., and a time of 5 hours.

FERMENTATION OF MALTED GRAINS

Malt is very efficient in the saccharification of starch containing grains. Optimum temperature for the malting of starch is said to be 55° C. and optimum pH, 5. In the series of experiments involving malting these conditions were used. The starch was first gelatinized and then malt equal to 10 per cent of the weight of the grain was added.

TABLE 2. *Influence of time, pressure and acid concentration upon the acid saccharification of Iodent corn*

(Percentage dry corn in acid solution = 13.1)

N. of HCl soln.	Yield glucose (basis dry grain)	N. of HCl soln.	Yield glucose (basis dry grain)
(Cooked 1 hr. Atm. pressure)		(Cooked 1 hr. 25 lbs. pressure)	
0.0	1.2	0.0	1.2
0.1	14.4	0.03	15.8
0.2	30.6	0.06	59.6
0.3	46.0	0.10	76.4
0.4	59.5	0.15	77.5
0.5	64.1	0.20	80.5
0.6	65.0	0.25	82.5
0.7	65.9	0.30	80.5
0.8	74.4	0.40	78.5
0.9	70.2		
(Cooked ½ hr. 20 lbs. pressure)		(Cooked 2 hrs. 25 lbs. pressure)	
0.05	17.7	0.03	23.9
0.10	50.3	0.06	75.8
0.20	78.5	0.10	81.5
0.30	78.5	0.15	80.0
0.50	78.5	0.20	80.0
0.70	82.8	0.25	78.2
0.90	82.1	0.30	75.4
1.10	80.1	0.40	73.5

TABLE 3. *Influence of time, pressure and acid concentration upon the acid saccharification of oats*

(Percentage dry oats in acid solution = 13.1)

N. of HCl soln.	Yield glucose (basis dry grain)	N. of HCl soln.	Yield glucose (basis dry grain)
(Cooked ½ hr. Atm. pressure)		(Cooked 1 hr. 10 lbs. pressure)	
0.0	1.2	0.2	60.5
0.1	1.5	0.4	61.8
0.2	13.8	0.6	59.7
0.3	22.3	0.8	58.6
0.4	29.0	1.2	57.2
0.5	31.8	1.6	49.4
0.6	38.8		
0.8	45.3		
1.0	47.0		
1.2	57.5		
1.6	59.0		
2.0	59.0		
(Cooked 1 hr. Atm. pressure)		(Cooked ½ hr. 20 lbs. pressure)	
0.1	8.1	0.1	38.8
0.2	29.5	0.3	64.0
0.3	37.0	0.4	64.0
0.4	47.4	0.5	63.5
0.5	51.5	0.6	64.9
0.6	53.6	0.8	62.8
0.8	58.6	1.0	62.8
1.2	60.0	1.2	61.2
1.6	60.0		
2.0	59.4		

TABLE 4. *Influence of time, pressure and acid concentration upon the acid saccharification of barley*

(Percentage dry barley in acid solution = 13.2)

N. of HCl soln.	Yield glucose (basis dry grain)	N. of HCl soln.	Yield glucose (basis dry grain)
(Cooked 1 hr. Atm. pressure)		(Cooked ½ hr. 20 lbs. pressure)	
0.2	27.5	0.0	0.8
0.3	41.4	0.2	58.4
0.4	52.4	0.3	70.0
0.5	56.0	0.4	72.3
0.6	68.6	0.6	72.5
0.7	69.5	0.8	75.0
0.9	69.9	1.0	73.5
1.0	69.9		
(Cooked 1 hr. 10 lbs. pressure)		(Cooked ½ hr. 20 lbs. pressure) Barley conc. = 8.62	
0.0	1.2	0.0	0.8
0.2	47.4	0.2	72.2
0.4	64.3	0.3	76.2
0.6	73.7	0.4	76.2
0.8	70.3	0.5	77.2
1.0	75.4	0.6	75.7
1.2	75.4	0.7	75.0
1.4	73.2	0.9	71.8
1.6	73.2	1.0	71.3
1.8	70.0		
2.0	70.0		

Using the above conditions the rate of diastatic action was determined, showing that the major part of the conversion occurred in the first 15 minutes after the malt infusion. Table 6 shows the maltose percentage at different times running up to 2 hours. If the malt is destroyed at this point the alcohol yield is decreased as certain of the more stable maltodextrins are acted upon throughout the fermentation.

The method used for determination of alcohol yields from the malted grains was the same as that on the fermented acid hydrolyzed liquors. Table 7 gives the various alcohol yields obtained.

Table 8 compares the optimum yields from the malted grains against those of the acid hydrolyzed mashes.

The action of malt after a 2 hour period at 55° C. may be seen from the following experiment. After malting for 2 hours, 3 flasks of each grain were heated to destroy the diastase, the flasks were then inoculated with a yeast culture and allowed to ferment. Another set of fermentations was treated in identical manner except the diastase was not destroyed after malting. Destruction of the diastase by heating reduced the yield from 29.8 per cent alcohol to 20.4 per cent, yields being calculated on the moist grain.

TABLE 5. *Alcohol yields from various grains with acid hydrolysis*

Barley (<i>Trebi</i>)			
Time (hrs.)	Pressure	N. HCl added to grain	Alcohol yield (basis dry grain)
1	Atm.	1.0	19.1
2	Atm.	1.0	19.8
2	Atm.	1.0	20.8
1½	20 lbs.	0.40	25.0
2	25 lbs.	0.15	24.7
3	25 lbs.	0.10	25.3
2	25 lbs.	0.10	20.5
2	25 lbs.	0.15	25.2
4	25 lbs.	0.05	9.5
5	25 lbs.	0.05	10.9
Wheat			
1	Atm.	1.0	17.9
1	Atm.	1.0	22.3
1½	20 lbs.	0.4	24.8
2	25 lbs.	0.15	25.2
2	25 lbs.	0.15	26.0
2	25 lbs.	0.10	22.3
3	25 lbs.	0.10	25.8
3	25 lbs.	0.05	14.6
5	25 lbs.	0.05	15.5
Oats			
1	Atm.	1.0	15.4
2	Atm.	1.0	15.9
2	Atm.	1.0	16.8
1½	20 lbs.	0.4	17.9
2	25 lbs.	0.15	11.3
2	25 lbs.	0.15	17.7
2	25 lbs.	0.10	14.2
3	25 lbs.	0.10	17.9
4	25 lbs.	0.05	3.3
5	25 lbs.	0.05	2.9
Corn (Iodent)			
1	Atm.	1.0	22.4
2	Atm.	1.0	12.6
2	Atm.	1.0	25.1
1½	20 lbs.	0.2	17.2
2	25 lbs.	0.15	29.7
2	25 lbs.	0.10	28.8
2	25 lbs.	0.10	30.7
3	25 lbs.	0.10	30.6
4	25 lbs.	0.05	27.4
5	25 lbs.	0.05	29.7

TABLE 6. *Malting time of corn*

Malting temperature—55° C. Conc. of corn—15 per cent. Conc. of malt—10 per cent (by wt. of corn)	
Time (min.)	Reducing sugar as maltose
0	3.0
5	21.5
15	35.0
25	41.0
35	43.3
60	46.1
90	48.4
120	50.6

*TABLE 7. *Alcohol yields from malted grains*

Grain	Per cent alcohol (basis dry grain)	Grain	Per cent alcohol (basis dry grain)
Wheat	29.4	Krug corn	32.0
Wheat	28.0	"401" corn	31.5
Iodent corn	34.4	"426" corn	27.0
Iodent Corn	35.8	Barley	24.1
Iodent corn	35.2	Oats	22.5
Iodent corn	34.3	Oats	22.9
Iodent corn	34.3		

* Different values for a given grain indicate more than one set of data.

TABLE 8. *Comparison of optimum yields of alcohol from various grains using acid and malt for saccharification*

Grain	Yield of alcohol (dry basis grain)	
	Malt saccharification	Acid saccharification
Corn	34.0	30.7
Oats	22.9	17.9
Barley	24.1	25.3
Wheat	29.4	26.0

SUMMARY AND CONCLUSIONS

Optimum conditions were determined for the acid saccharification of various grains from the standpoint of final reducing sugar concentrations and resulting yields of alcohol from the fermented acid hydrolyzed liquors.

Maximum alcohol yields were determined using both acid saccharification and malting of the different grains, the latter giving the higher yields.

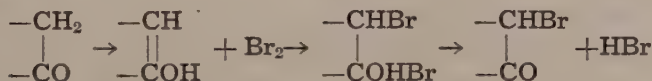
THE BROMINATION OF FURYL METHYL KETONE

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The great tendency of furan to undergo nuclear substitution when treated with a reagent which can effect furan nuclear substitution has been demonstrated in the case of ethyl furylacrylate¹ and 2-furyl phenyl ketone². The only exception has been the addition of bromine to the side chain of furyl ethylene³. In the bromination of furyl methyl ketone it has been shown that the bromine enters the side chain to give *w*-bromo-furyl methyl ketone. This behavior may be explained by the theory of C. F. Ward⁴. This theory assumes enolization of the carbonyl group and addition to the unsaturated linkage with a final removal of hydrogen bromide.



It was expected, however, that the second atom of bromine would substitute in the nucleus. This was shown not to be the case as the dibromination of furyl methyl ketone gives *w,w*-dibromofuryl methyl ketone in 90 per cent yield. It was thought that this formation of a chain substituted compound was caused by the removal of hydrogen bromide from an addition product which may have contained bromine attached to the nucleus. To test this theory the bromination was carried out at low temperature and the product treated with alcoholic potassium hydroxide before it reached a point where hydrogen bromide was given off spontaneously. However, a careful examination of the reaction product failed to show a compound containing a bromine atom in the nucleus.

The nitration product of furyl methyl ketone⁵ has been shown to be 5-nitro-2-furyl methyl ketone by comparison with the product from the reaction of diazomethane with 5-nitro-2-furfural⁶. This ketone has been characterized by the preparation of the oxime.

EXPERIMENTAL

MONOBROMINATION OF FURYL METHYL KETONE

To 11 g. (0.8 mole) of furyl methyl ketone dissolved in 100 cc. of carbon disulfide (dried over calcium chloride), 16 g. (0.1 mole) of bromine

¹ Gilman and Wright, *J. Am. Chem. Soc.*, **52**, 3349 (1930).

² Gilman and Young, *J. Am. Chem. Soc.*, **56**, 464 (1934).

³ Moureu, Dufraisse and Johnson, *Ann. chim.*, **7**, 8 (1927).

⁴ C. F. Ward, *J. Chem. Soc.*, **123**, 2207 (1923).

⁵ Rinkes, *Rec., trav. chim.*, **51**, 349 (1932).

⁶ Gilman and Wright, *J. Am. Chem. Soc.*, **52**, 2250 (1930).

in 500 cc. of carbon disulfide was added. This bromine was added dropwise with stirring at room temperature. The mixture was stirred 15 minutes after the last addition of bromine. It was then poured into water and washed with sodium bicarbonate solution. The carbon disulfide layer was dried and most of the carbon disulfide removed. The residue was distilled under reduced pressure. The fraction boiling 120-5° C. at 20 mm. pressure was collected and redistilled. The yield of redistilled *w*-bromofuryl methyl ketone b.p. 121-3° C. at 20 mm. pressure was 16 g. This was 90 per cent of the theoretical amount. The product thus obtained was a liquid and would not solidify on cooling. Other constants are:

N_D^{25} 1.5783; D_4^{25} 1.5785.

MR_D : Calcd 36.57, Obs. 39.75

However, if this oil were dissolved in petroleum ether at room temperature and then vigorously cooled, crystals were deposited which after two crystallizations melted constantly at 36-7° C.

Anal. Calcd. for $C_6H_5O_2Br$: Br, 42.33

Found: Br, 42.26. 42.21

The above run was repeated except that the temperature was kept below -15° during the reaction and during the stirring after the reaction. No hydrogen bromide was evolved. A portion of the product was treated with alcoholic sodium hydroxide at 0° C. Another portion was treated with pyridine and the rest was allowed to come up to room temperature and evolve hydrogen bromide. From none of these fractions was any compound isolated which contained nuclear bromine.

OXIDATION OF *w*-BROMOFURYL METHYL KETONE

Two grams (0.01 mole) of *w*-bromofuryl methyl ketone and 4 g. of calcium hydroxide were suspended in ice water and 1 g. of potassium permanganate in water was added. The mixture was heated to boiling and filtered. The solution was then acidified and extracted with ether, yielding 0.4 g. of furoic acid. This was 35 per cent of the theoretical amount. The furoic acid was identified by its melting point of 129-30° C. and a mixed melting point with an authentic sample showed no depression.

Three grams (0.015 mole) of *w*-bromofuryl methyl ketone were treated with 3 cc. of pyridine in 50 cc. of dry ether and refluxed one hour. The reaction mixture was cooled and a layer of gummy material separated. A portion of this gummy material which was undoubtedly furacyl pyridinium bromide was then dissolved in water and treated with sodium hydroxide at 40°. The solution was then acidified and extracted with ether, giving furoic acid. The furoic acid was identified by its melting point, 129-130° C. and by mixed melting point with an authentic sample.

w-BROMOFURYL METHYL KETONE FROM THE FRIEDEL-CRAFTS REACTION

To 30 g. (0.24 mole) of aluminum chloride in 50 cc. of carbon disulfide, 41 g. (0.2 mole) of bromacetyl bromide in 100 cc. of carbon disulfide were added at room temperature. Fourteen grams (0.2 mole) of furan in 50 cc. of carbon disulfide were added to this mixture. It was stirred

for 15 minutes after the addition and then poured into ice-water to decompose it. The carbon disulfide layer was washed several times with sodium bicarbonate solution, dried, and the solvent was removed. The residue was distilled and the fraction boiling at 122-5° C. at 20 mm. was collected. The yield of *w*-bromofuryl methyl ketone was 7 g. This was 38 per cent of the theoretical amount. The oily product was crystallized from petroleum ether. M.P. 36-37° C. It was shown to be the same compound as that derived from the bromination of feryl methyl ketone as there was no depression of the melting point when the two were mixed.

DIBROMINATION OF FURYL METHYL KETONE

Thirty-two grams (0.2 mole) of bromine in 50 cc. of carbon disulfide were added dropwise with stirring at room temperature to 11 g. (0.1 mole) of feryl methyl ketone. When the hydrogen bromide evolution ceased the reaction mixture was poured into water, washed with sodium bicarbonate solution, dried, and the solvent removed. The residue was distilled under reduced pressure. The fraction boiling at 140-150° C. at 15 mm. pressure was collected. On refractionation the fraction boiling at 145-147° C. at 15 mm. pressure was collected. The yield of *w,w'*-dibromofuryl methyl ketone was 24.5 g. This was 90 per cent of the theoretical amount. Other constants are:

N_D^{25} 1.6070. D_4^{25} 2.0040

M.R. Calcd. 76.2, Obs. 81.2

Anal. Calcd. for $C_6H_4O_2Br_2$: Br, 59.70

Found: Br, 59.30, 59.37.

OXIDATION OF *w,w'*-DIBROMOFURYL METHYL KETONE

Five grams of the ketone were refluxed in ether with 5 cc. of pyridine. No salt separated on cooling so the ether solution was extracted with water and the water extract treated with sodium hydroxide at 40° C. The solution was acidified and extracted with ether, giving 0.5 g. of furoic acid m.p. 129-130° C. This was 20 per cent of the theoretical amount. The acid was identified by a mixed melting point with an authentic sample and showed no halogen test on fusion with sodium.

Five grams (0.02 mole) of the ketone and 10 g. of calcium hydroxide were suspended in ice water and treated with 2.5 g. of potassium permanganate in solution. The mixture was heated, acidified and extracted with ether, giving 1.4 g. of acid. This was 65 per cent of the theoretical amount of furoic acid melting point 125-127° C. The furoic acid was identified by mixed melting point with an authentic sample and showed no halogen test on fusion with sodium.

All the other fractions from a dibromination run were submitted to oxidation in the above manner but no trace of a compound containing nuclear halogen was found.

BROMINATION OF 5-BROMOFURYL METHYL KETONE

Ethyl 5-bromofuroate was converted to ethyl 5-bromofuroyl acetate by the Claisen condensation in 34 per cent yield. The ethyl 5-bromofuroyl acetate was hydrolyzed by dilute sulfuric acid in 80 per cent yield,

giving 5-bromofuryl methyl ketone. To 30 g. (0.16 mole) of 5-bromofuryl methyl ketone in 100 cc. of carbon disulfide, 24 g. (0.15 mole) of bromine were added at room temperature with stirring. The mixture was stirred until the hydrogen bromide evolution ceased and it was then poured into water. The carbon disulfide was washed with sodium bicarbonate solution, dried, and the solvent removed. The residue was distilled under reduced pressure. The fraction boiling 150-155° C. at 19 mm. pressure was collected. It solidified in the receiver and was then recrystallized to the constant melting point of 98.5-99.5° C. The yield was 21 g. of *w*,5-dibromofuryl methyl ketone. This was 50 per cent of the theoretical amount.

Anal. Calcd. for $C_6H_4O_2Br_2$: Br, 59.70

Found: Br, 59.91, 59.85.

OXIDATION OF *w*, 5-DIBROMOFUYRL METHYL KETONE

To 1 g. (0.004 mole) of the ketone and 2 g. of calcium hydroxide suspended in ice water 0.6 g. of potassium permanganate was added in solution. The reaction mixture was heated, acidified, and extracted with ether, giving 0.25 g. of 5-bromofuroic acid. This was 36 per cent of the theoretical amount. The acid on recrystallization melted at 184° C. and a mixed melting point with an authentic sample showed no depression.

To 1 g. (0.004 mole) of the ketone 1 cc. of pyridine in ether was added. The mixture was refluxed one hour and filtered. The residue was dissolved in water, treated with sodium hydroxide at 40° C., acidified, and extracted with ether, yielding 0.5 g. of acid. This was 70 per cent of the theoretical amount of 5-bromofuroic acid. This acid was identified by its melting point and by a mixed melting point with an authentic sample.

5-NITROFUYRL METHYL KETONE

5-Nitrofuryl methyl ketone was prepared by the method of Rinkes. This compound was shown to be 5-nitro-2-acetylfuran by the following reaction. Diazomethane in ether was prepared by heating 45 g. (0.8 mole) of potassium hydroxide in 200 cc. of methyl alcohol with 25 g. (0.2 mole) of nitrosomethyl urethane in 300 cc. of ethyl ether and distilling the mixture of diazomethane and ether. To this solution 14.1 g. (0.1 mole) of 5-nitrofurfural were added. A rapid evolution of nitrogen took place and, after the completion of the reaction, the ether was removed and the product crystallized. The melting point of 5-nitro-2-acetylfuran was 78-78.5° C. When mixed with the nitrofuryl methyl ketone of Rinkes it was 78-78.5° C.

5-NITROFUYRL METHYL KETOXIME

Because of the sensitivity of the furan nitro group to alkali, this oxime was prepared in acid solution. To 5 g. (0.03 mole) of 5-nitrofuryl methyl ketone and 4 g. (0.06 mole) of hydroxylamine hydrochloride in alcohol solution one-half cc. of concentrated hydrochloric acid was added. The mixture was heated to 100° C. for 2 hours in a closed vessel. The yield of 5-nitrofuryl methyl ketone, melting point 167-168° C., was 5.1 g. This was 93 per cent of the theoretical amount.

SUMMARY

1. It has been shown that in the bromination of 2-furyl methyl ketone the first bromine enters the side chain. It has been further shown that the second bromine will also enter the side chain.
2. In the bromination of 5-bromo-2-furyl methyl ketone the bromine enters the side chain.
3. The nitrofuryl methyl ketone prepared by Rinkes has been shown to be 5-nitro-2-furyl methyl ketone.

THE OXIDATION OF FURAN METHYL GROUPS

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Although oxidation in the furan series has been studied since 1873, when Limpricht¹ worked with furoic acid, no general method and few special methods have been reported for the oxidation of furan compounds without decomposition of the furan ring. Some of the agents that have been used for the oxidation of furan compounds to aliphatic acids such as fumaric, succinic, oxalic, etc., are: aqueous bromine², dilute nitric acid³, potassium permanganate⁴, peracetic acid⁵, sodium chlorate and vanadium pentoxide or osmium tetroxide⁶, air and vanadium trioxide, vanadium pentoxide, or ammonium vanadate⁷, oxygen and vanadium pentoxide⁸, Caro's acid⁹, and many others. There are a number of isolated cases of oxidation in which the furan ring is kept intact, but only a few will be enumerated. Boeseken¹⁰ and his coworkers using 70 per cent peracetic acid in acetic acid solution, have obtained what they consider to be an oxide of furan. Furfural and other furan aldehydes have been oxidized to the corresponding acids by potassium permanganate¹¹, silver oxide², air¹², and other agents. The Cannizzaro reaction goes smoothly with a number of furan aldehydes. Furoin has been oxidized to furil by air¹³ or electrolysis¹⁴. Moureu, Dufraisse, and Johnson¹⁵ have reported the supposed oxidation of furylbromoethylene with air to *w*-bromofuryl methyl ketone. Priebs¹⁶ has oxidized 5-nitro-2-ethenyl nitro-furan to 5-nitro-2-furoic acid with chromic acid. Gilman and Wright¹⁷ have oxidized 5-nitro-furfuryl alcohol to nitrofurfural by heating to 40-50° for two days with manganese dioxide and 50 per cent sulfuric acid.

¹ Limpricht, *J. Chem. Soc.*, 26, 624 (1873).

² Hill and Jennings, *Proc. Am. Acad.*, 27, 186 (1891).

³ Hill and Hartshorn, *Ber.* 18, 448 (1885).

⁴ Atterberg, *J. Chem. Soc.*, 40, 663 (1881).

⁵ Boeseken and coworkers, *J. Chem. Soc.*, (T) 75, 747 (1899).

⁶ Milas, *J. Am. Chem. Soc.*, 49, 2005 (1927).

⁷ Boehringer, *British Pat.*, 297667.

⁸ Sessions, *J. Am. Chem. Soc.*, 50, 1696 (1928).

⁹ Cross and coworkers, *Ber.*, 31, 43 (1898); *Chem. News*, 82, 1631 (1900).

¹⁰ Boeseken and coworkers, *Rec. trav. chim.*, 50, 1023 (1931).

¹¹ (a) Volhard, *J. Chem. Soc.*, 60, 896 (1891); *Ann.*, 261, 379 (1895).

(b) Gilman and Wright, *Rec. trav. chim.*, 50, 833 (1931).

¹² Moureu, Defraisse, and Lotte, *Comp. rend.*, 180, 993 (1925).

¹³ E. Fisher, *J. Chem. Soc.*, 40, 798 (1880).

¹⁴ Law, *J. Chem. Soc.*, 89, 1445 (1906).

¹⁵ Moureu, Dufraisse, and Johnson, *Ann. Chim. phys.*, 7, 14 (1927).

¹⁶ Priebs, *Ber.*, 18, 1362 (1885).

¹⁷ Gilman and Wright, *J. Am. Chem. Soc.*, 53, 1923 (1931).

Methyl groups in the furan series have been oxidized¹⁸ by bromination to give the dibromide followed by hydrolysis to the aldehyde. The drastic conditions of this method, however, keep it from being general.

The oxidizing agent used in these studies is one that has been applied successfully by W. A. Noyes¹⁹ to the benzene series, and appears to be general for the oxidation of furan methyl groups to carboxylic acids. This reagent, potassium ferricyanide, combines power with mildness in a way which makes it an ideal oxidizing agent in the furan series. With it such compounds as furfuryl alcohol and sylvan have been oxidized to furoic acid.

EXPERIMENTAL

TYPICAL OXIDATION FOR MOST TYPES OF FURAN COMPOUNDS

Oxidation in basic solution may be used for all compounds except the nitrofurans which are unstable in the presence of alkalis. A typical oxidation is outlined.

One gram of the compound, 25 g. of potassium ferricyanide, and 10 g. of potassium hydroxide were placed in a 300 cc. flask with 150 cc. of distilled water and refluxed three hours. The solution was filtered hot to remove iron oxide, and concentrated to one-half the original volume. On cooling a large quantity of potassium ferrocyanide crystallized and was filtered. This potassium ferrocyanide could be converted to potassium ferricyanide and used again. The filtrate was then acidified with hydrochloric acid in slight excess of that required to neutralize the base. It was not necessary to use enough hydrochloric acid to convert the potassium ferrocyanide to the acid as hydroferrocyanic acid was stronger than the organic acids prepared. The acid solution was extracted with ether. Removal of the ether left the impure organic acid which was purified by crystallization. In some cases where the yields were poor it was found advantageous after boiling two hours to add another 25 g. of potassium ferricyanide and 10 g. of potassium hydroxide and continue the boiling another two hours. Table 1 gives the amounts of reagents used and the yields of acid in grams.

OXIDATION OF GROUPS IN FURAN NITRO COMPOUNDS

In the case of the furan nitro compounds, two equivalents, 34 g. of potassium acetate, were used instead of the 10 g. of potassium hydroxide. The procedure was the same as before with this exception. The neutral type of reaction, using potassium acetate, was also tried with some of the other furan compounds.

¹⁸ Hill and Sawyer, *Am. Chem. J.*, **20**, 169 (1898).

¹⁹ (a) W. A. Noyes, *Am. Chem. J.*, **5**, 97 (1883); (b) **7**, 145 (1885); **8**, 176 (1886); (d) **9**, 93 (1887); (e) **10**, 472 (1888); (f) **11**, 161 (1889).

TABLE 1.

Compounds oxidized	Sample grams	Grams potassium ferri-cyanide used	Acid obtained	Yield grams
Sylvan	1	50	furoic	.05
Dimethyl furan	1	25	dehydromucic	.01
Furyl methyl ketone	1	25	furoic	.51
Furylacrylic acid	1	25	not oxidized	
5-Methyl-2-furoic	1	25	dehydromucic	.35
Furfural	1	25	furoic	.22
5-Bromofuryl methyl ketone	1	25	5-bromofuroic	.45
Furyl methyl ketone ¹	1	25	furoic	.30
5-Nitrosylvan ¹	1	25	5-nitrofuroic	.54
Furfuryl alcohol	1	25	furoic	.21
Furfural acetone	1	25	furylacrylic	.10
Furfural acetone ¹	1	25	furylacrylic	.10
Furil	$\frac{1}{2}$	25	furoic	.41
2-Methyl-3-furoic acid	1	75	2,3-dicarboxyfuran	.35
Tertiarybutyl furoic acid	$\frac{1}{2}$	50	dehydromucic	.01
Furyl ethylene	1	25	furoic acid	.02

¹ All of these were run in neutral solution.

The acids were characterized by m.p. and mixed m.p. with authentic samples.

In the case of dehydromucic acid, which has no melting point, the acid was converted to the dimethyl ester and a m.p. and a mixed m.p. were taken.

SUMMARY

1. An oxidizing agent which seems to be general for the oxidation of furan methyl groups has been reported. A number of other furan compounds have been oxidized using the same reagent.

THE AVAILABILITY OF PHOSPHORUS IN SOME IOWA SOILS¹

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The phosphorus content of most Iowa soils, according to the results of analyses of all types mapped in the state, is quite low. The amount present has actually been found to vary from as low as 0.015 per cent up to 0.12 per cent, the average figure for 1,345 analyses of samples of the various types being 0.0657 per cent. But the total amount of phosphorus present in the soil often bears no relation to the amount available for plant use. Hence, much importance is attached to the problem of the determination of the available phosphorus content of soils and its relationship to the total supply. Many methods have been proposed for the estimation of the available phosphorus in soils but none has yet been devised which measures accurately the amount present in all soils. The best method now available is still the laborious and time-consuming determination of crop responses to phosphate fertilizers in field experiments.

The object of the present investigation was to determine the available phosphorus content of a number of Iowa soils using various methods and to compare the results with the crop response to phosphate fertilizers in field tests. It was also hoped that the data would throw some light on the problem of the factors influencing availability.

The 1 per cent citric acid method proposed by Dyer (2) in 1894 marks the first important use of a weak acid solvent for determining the available phosphorus in soils. Since the work of Dyer many other methods using various dilute acids have been proposed (1), (5), (16), (21).

Von Wrangell (22) and McGeorge (8) have studied the rate of solution of phosphorus in the soil, the former by water extraction and the latter by electrodialysis, in an attempt to determine the availability of the phosphorus. Hibbard (5) suggested a modification of the von Wrangell procedure and Fisher and Thomas (3) proposed the use of two solvents to differentiate various forms of phosphorus in the soil.

Truffaut and Bezssonoff (20) advocated the use of a mixed culture of *Clostridium pasteurianum*, *Bacillus triffauti* and *Azotobacter agile* to measure available phosphorus. This method is based on the assumption that since bacteria are plants and require phosphorus for their normal growth and development, their requirements will represent those of higher plants. Certain species of these bacteria living in the soil in the presence of an abundant supply of phosphorus are able to decompose sugar and secure from the air the nitrogen which they need for growth. The amount of nitrogen fixed by these bacteria is proportional to the growth they make and the growth made is proportional to the amount of phosphorus the bacteria are able to secure from the soil. The amount of nitrogen fixed by a culture of these bacteria in a medium with the soil in question as the source

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of phosphorus is regarded, therefore, as a measure of the available phosphorus.

Niklas et al (11) found that *Aspergillus niger* made more growth in a solution well supplied with phosphorus than in a solution poor in this element. They claim that the amount of growth made in a soil suspension by this mold is proportional to the amount of available phosphorus in that soil.

Another test (9) similar to the *A. niger* method depends upon the development of a mold of the genus *Cunninghamella* when seeded on the surface of the soil in small glass dishes. The assumption is also made in this test that the development of the mold is proportional to the available phosphorus present in the soil.

Neubauer (10) proposed the seedling plant method for determining the available phosphorus in the soil. This method consists of planting 100 rye seeds in a small glass dish of the soil to be tested and with proper temperature and moisture conditions it is claimed the rye seedlings will grow until they exhaust the food supply of the soil. The seedlings are then analyzed for phosphorus and the amount found is supposed to represent the amount of available phosphorus in the soil. Both favorable (19) and unfavorable (13) results have been reported from the use of this method.

These methods are all based upon certain assumptions and this brief review shows that not all of the assumptions made are valid. However, a certain degree of success has been attained by the use of the various methods and certainly much information concerning the factors affecting phosphorus availability has been secured.

METHODS OF PROCEDURE

1. Cooperative Soil Experiment Fields

Samples of soils were collected in the fall of 1933 from the untreated plots, the limed plots and the lime plus rock phosphate treated plots from 8 of the cooperative soil experiment fields and from the untreated and the rock phosphate treated plots of the Everly field experiment (table 1). The crop yields obtained on all these plots from 1924-1933 were averaged and are shown in table 2. The soils were brought into the laboratory, air-dried and passed through the 2 mm. sieve. The air-dry soils were thoroughly mixed and small representative samples were ground to pass the 100-mesh sieve for the analyses.

The pH of the unground soils was determined on the air-dry samples by the quinhydrone electrode. The lime requirement was determined on the ground sample according to the procedure recommended by Hardy and Lewis (4). The total phosphorus content of the soils was determined by the magnesium nitrate method. The results of these tests are shown in table 3.

2. Biological Methods

The availability of the phosphorus in these soils was determined by four biological methods, viz.: the bacteriological method of Truffaut and Bezssonoff (20), the *A. niger* method of Niklas et al (11), the *Cunninghamella* method (9), and the Neubauer method (10). In the use of the bacteriological method certain modifications were necessary. *B. truf-*

TABLE 1. Outline of field soil treatment

Soil No.	Soil type	Field	Treatment*
1	Carrington loam	Waverly	O
2	" "	"	L
3	" "	"	LRP
4	Carrington silt loam	Springville	O
5	" " "	"	L
6	" " "	"	LRP
7	Grundy silt loam	Agency	O
9	" " "	"	L
10	" " "	"	LRP
11	Grundy silty clay loam	West Union	O
13	" " "	" "	L
14	" " " "	" "	LRP
15	Lamoure silty clay loam	Everly	O
16	" " " "	"	RP
17	Marshall silt loam	Red Oak	O
19	" " "	" "	L
20	" " "	" "	LRP
21	Muscatine silt loam	Letts	O
23	" " "	"	L
24	" " "	"	LRP
25	O'Neill loam	Everly	O
26	" "	"	L
27	" "	"	LRP
28	Tama silt loam	Hudson	O
29	" " "	"	L
30	" " "	"	LRP

* O = No treatment

L = Lime

LRP = Lime + rock phosphate

fauti was not available and this organism was eliminated from the culture. *Azotobacter chroococcum* was used instead of *Az. agile*.

3. Chemical Methods

These methods are based upon the assumption that the reagents used will dissolve out only the phosphorus available to the plants and no more. A number of weak acids or dilute solutions of strong acids have been used for this purpose. Most of the chemical methods involve the use of the Denigés colorimetric method for the determination of the phosphorus. The differences in the methods are, for the most part, differences in the manner of extraction and in the extracting solution. In this work the Bray (1) test using hydrochloric acid, the Truog (21) test using 0.002N sulfuric acid, the Dyer (2) 1 per cent citric acid method and 1 per cent acetic acid were employed. In addition to these solutions hydrochloric acid solutions of 0.05 and 0.005 N concentrations were used. A saturated solution of carbon dioxide at 25° C. and 50 mm. of water pressure was used, Fig. 1. The procedure followed in the determinations by hydrochloric and carbonic acid was the same as that followed in the Truog method except that these acids were used instead of sulfuric acid. Calcium

TABLE 2. *Average yields of crops on outlying field experiments*
(10-year average, 1924-1933, inclusive)

Soil No	Soil Type	Treatment	Corn Bu/A	Oats Bu/A	Winter Wheat Bu/A	Soy beans Bu/A	Soy beans tons/A	Clover, mixed clover hay tons/A	Alfalfa tons/A
1	Carrington loam	O	39.3**	38.5 ²			0.42 ²		
2	" "	L	51.3	47.6			1.53		
3	" "	LRP	51.8	59.1			1.81		
4	Carrington silt loam	O	49.3 ⁴	42.9 ⁴			0.73		
5	" "	L	48.2	47.7			1.05		
6	" "	LRP	43.3	56.2			1.41		
7	Grundy silt loam	O	61.4 ⁴	61.5 ²	20.0 ²		1.59 ²		
9	" "	L	68.1	65.8	23.5		1.92		
10	" "	LRP	74.1	72.4	28.5		2.23		
11	Grundy silty clay loam	O	58.5 ⁶	39.2	16.7	20.3			
13	" "	L	58.1	30.9	14.0	19.2			
14	" "	LRP	59.2	39.6	16.0	18.9			
15	Lamoure silty clay loam	O	52.1 ⁴	53.4					1.86 ⁴
16	" "	RP	55.9	72.4					2.71
17	Marshall silt loam	O	65.0 ²		8.6	9.9			3.39 ⁴
19	" "	L	66.5		10.2	13.2			3.56
20	" "	LRP	62.9		13.0	12.3			3.71
21	Muscataine silt loam	O	66.7 ⁸	57.4 ²	19.1 [*]			0.50	
23	" "	L	64.1	59.1	20.1 [*]			0.75	
24	" "	LRP	74.9	60.4	26.3 [*]			1.32	
25	O'Neill loam	O	40.9 ⁵	44.0 ²				0.43	1.41 ²
26	" "	L	40.6	52.0				0.36	1.40
27	" "	LRP	50.0	49.4				0.41	1.53
28	Tama silt loam	O	48.1 ⁵	46.9 ²				1.69	
29	" "	L	56.7	53.4				2.27	
30	" "	LRP	59.8	60.7				2.32	

* Barley.

** Numbers above and to the right of the figure refer to number of crops.

TABLE 3. *Reaction, lime requirement and total phosphorus content of soils*

Soil No.	Treatment	pH	Lime requirement tons per acre Hardy-Lewis method	Pctg. total phosphorus (oven-dry soil)
1	O	5.40	2.5	0.062
2	L	6.28	0.8	0.060
3	LRP	6.90	0.7	0.103
4	O	5.34	4.7	0.060
5	L	6.78	1.2	0.056
6	LRP	6.66	1.1	0.080
7	O	5.26	4.5	0.056
9	L	4.93	2.7	0.062
10	LRP	5.72	1.5	0.090
11	O	6.36	1.4	0.077
13	L	6.13	1.0	0.066
14	LRP	5.78	1.0	0.103
15	O	8.22	0.070
16	RP	8.24	0.109
17	O	5.84	2.2	0.070
19	L	6.40	1.0	0.064
20	LRP	6.48	1.0	0.079
21	O	5.10	4.6	0.064
23	L	6.04	1.3	0.060
24	LRP	5.73	1.4	0.071
25	O	6.06	1.9	0.065
26	L	6.65	0.8	0.068
27	LRP	6.98	1.0	0.073
28	O	5.36	4.0	0.054
29	L	6.88	1.1	0.053
30	LRP	6.90	1.0	0.078

and iron were determined in the extracts of 0.05 and 0.005 N hydrochloric acid.

4. *Effect of Lime and Aluminum Sulfate on the pH and Availability of Phosphorus in the Soil*

Each of the 30 soils was treated in duplicate in 3 series as follows:

Series 1—Check

Series 2—CaCO₃ in the amount of 4 times the lime requirement

Series 3—1 per cent aluminum sulfate

The soils were treated in tumblers, the moisture was adjusted to 25 per cent and maintained at this amount by frequent additions of distilled water. After 51 days the soils were air-dried and the pH and phosphorus soluble in 0.002 N H₂SO₄ determined.

RESULTS

The average crop response to phosphorus additions varied on the different soils with the crop grown, as appears in table 2. For example, corn did not show any significant response to phosphorus on the Carrington loam and the Carrington silt loam, but with oats and soybeans there was considerable response to phosphorus on these soils. On the other

TABLE 4. Available phosphorus by biological methods

Soil No.	Treatment	p.p.m. available P bacteriological method	Mgm. <i>A. niger</i> mycelium	p.p.m. available P Cunninghamella method	Mgm. P ₂ O ₅ Neubauer method
1	O	19.0	90.2	12.5	2.5
2	L	116.9	1.5	1.7
3	LRP	76.5	250.3	trace	7.1
4	O	80.1	11.5	0
5	L	75.0	3.5	3.3
6	LRP	233.6	5.5	1.1
7	O	55.5	66.3	45.0	5.0
9	L	53.9	91.0
10	LRP	189.0	173.5	100.0
11	O	17.0	64.7	100.0	4.1
13	L	82.8	100.0	6.2
14	LRP	188.5	181.5	100.0	5.2
15	O	225.5	126.9	trace	0.6
16	RP	52.0	123.1	6.6
17	O	21.0	103.7	17.5	1.9
19	L	111.6	6.5
20	LRP	333.5	190.6	20.0
21	O	177.5	88.2	20.0	6.9
23	L	227.5	120.4	trace
24	LRP	161.5	161.6	8.5
25	O	160.5	119.7
26	L	108.5	130.5	trace	23.5
27	LRP	129.5	180.3	trace	19.7
28	O	169.5	82.3	15.0
29	L	237.5	80.7
30	LRP	70.0	153.2	trace

hand, corn showed a marked response to phosphorus on the Muscatine silt loam and the O'Neill loam, but with oats there was little or no effect of the phosphorus. No better explanation of these data apparently can be offered than the fact that the phosphorus exists in these soils in different forms and the different crops vary in their ability to assimilate the phosphorus from the existing forms. It is possible that nitrogen or potassium may be the limiting factor for corn on the Carrington loam but this does not seem probable in view of the yields of oats and other crops on this soil.

The reaction of all the untreated soils was acid, except the Lamoure silty clay loam. The pH of all the untreated soils was below 6.0, except with the O'Neill loam in which the pH was 6.06. The pH of the limed soils varied from 4.93 in the Grundy silt loam to 6.90 in the Tama silt loam. The total phosphorus content varied from 0.053 per cent in the limed Tama silt loam to 0.109 per cent in the phosphate treated Lamoure silty clay loam. The reaction and total phosphorus in the soils 4, 5 and 6, from the Springville field, and soils 28, 29 and 30 from the Hudson field, respectively, were quite similar.

The data in table 4 indicate considerable difference in the amount of available phosphorus in the different soils. However, there was no relation between the amount of available phosphorus in any one soil as meas-

ured by the various methods. According to the bacteriological method, the Marshall silt loam contained the largest amount of available phosphorus but this soil gave no response to phosphate fertilizer on corn and only small increases with other crops, such as wheat, soybeans and alfalfa. By the *A. niger* test this soil was third highest in available phosphorus and contained considerably more available phosphorus than the same soil untreated or that treated with lime. According to the Cunninghamella and Neubauer methods the Marshall silt loam contained only a small amount of available phosphorus. In general, the soils treated with phosphate fertilizers contained larger amounts of available phosphorus than the same soils untreated or those treated with lime. However, this was not true for all soils nor was it shown consistently by any one method.

The data in table 5 show that not one of the five chemical methods employed indicated accurately the phosphorus needs of the soil as measured by crop response to phosphorus fertilizers. The simple qualitative test employing HCl was probably the best indicator for all soils and the carbonic acid extraction was probably the most consistent quantitative

TABLE 5. Available phosphorus by chemical methods

Soil No.	Treatment	HCl* (Bray)	0.002 N H ₂ SO ₄	1% citric acid	1% acetic acid	H ₂ CO ₃
			p.p.m.	p.p.m.	p.p.m.	p.p.m.
1	O	+	5.21	207.0	1.59
2	L	+++	5.92	238.0	3.18	22.8
3	LRP	++++	199.80	87.40	55.6
4	O	+	5.38	92.0	1.09	18.1
5	L	+++	5.47	25.0	3.84	10.0
6	LRP	++++	118.90	258.0	77.20	52.0
7	O	+	7.59	2.71	21.6
9	L	+++	11.12	4.51	25.3
10	LRP	++++	66.80	212.0	121.30	56.5
11	O	++++	82.99	115.0	71.30	16.7
13	L	++++	40.14	92.0	81.30	16.7
14	LRP	++++	24.75	402.0	83.50	45.7
15	O	++++	24.75	51.0	47.50	37.3
16	RP	++++	25.45	554.0	65.80	23.4
17	O	++++	37.96	11.5	29.00	33.1
19	L	++++	27.10	40.2	22.10	28.4
20	LRP	++++	114.69	157.0	86.60	37.1
21	O	++	13.73	224.0	5.88	28.5
23	L	+++	32.42	379.0	27.90	22.4
24	LRP	++++	120.36	232.0	85.80	56.2
25	O	+++	17.03	299.0	17.50
26	L	+++	9.21	115.0	3.20	11.7
27	LRP	++++	72.21	350.0	42.50	14.6
28	O	+	10.46	117.0	1.11	20.3
29	L	+++	8.55	103.0	1.89	23.5
30	LRP	++++	69.11	35.30	30.9

* + Doubtful
 ++ Low
 +++ Medium
 ++++ High

TABLE 6. *Phosphorus, calcium and iron soluble in 0.05 N HCl*

Soil No.	Treatment	P in p.p.m.	Ca in p.p.m.	Fe in p.p.m.
1	O	21.2	3689	927
2	L	24.1	4918	1261
3	LRP	273.7	8607	1341
4	O	13.1	4099	1501
5	L	13.6	4099	1033
6	LRP	208.0	6558	1466
7	O	14.6	3689	650
9	L	14.7	4099	485
10	LRP	197.4	6558	700
11	O	183.5	10247	342
13	L	135.6	9427	420
14	LRP	274.7	9427	516
15	O	144.3	13116	1017
16	RP	312.0	20495	600
17	O	91.7	6558	630
19	L	64.1	6968	300
20	LRP	148.5	6968	49
21	O	16.3	4508	548
23	L	46.4	6968	346
24	LRP	104.0	4918	412
25	O	42.3	2869	864
26	L	29.3	5738	1001
27	LRP	152.9	7788	1168
28	O	11.1	3279	864
29	L	14.5	5738	941
30	LRP	114.2	7378	1190

measure in soils below pH 7.0. The variations in amounts of phosphorus soluble by this method appeared to be related to soil differences and especially to the pH of the soil and the presence of rock phosphate. The 1 per cent citric acid extraction gave higher results than the 0.002 N sulfuric acid in every case, except with soil No. 17. This was probably caused by a precipitation of the iron by the citric acid. The results obtained with the 1 per cent acetic acid extraction were similar to those with the 0.002 N sulfuric acid but somewhat lower in most soils.

The phosphorus, calcium and iron soluble in 0.05 N and 0.005 N HCl were determined in each of the soils. The data in tables 6 and 7 show that with the more concentrated HCl solutions the amount of soluble phosphorus was increased. The soils that contained large amounts of calcium were the soils that failed to show large amounts of soluble phosphorus with solvents other than HCl. The soluble iron content was high at low phosphorus solubility and low at high phosphorus contents in the Grundy silty clay loam, the Lamoure silty clay loam with 0.05 N HCl and the Carrington loam, the Carrington silt loam, the Lamoure silty clay loam, the Muscatine loam, and the O'Neill loam with the 0.005 N HCl.

The ratio of phosphorus soluble in 0.005 N HCl to that soluble in 0.05 N HCl was calculated and is shown in table 7. The nine soils may be divided into two groups on the basis of this ratio. The Grundy silty clay loam, the Lamoure silty clay loam, the Marshall silt loam, the Grundy

silt loam and the Muscatine silt loam belong in a group with an average ratio of 0.9. The Carrington silt loam, the Tama silt loam, the O'Neill loam and the Carrington loam are in a group with an average ratio of 0.4. In three of the five soils, where the ratio was 0.8 or above and of about the same magnitude in the limed and unlimed soils there was only little crop response to rock phosphate. On the other hand, there was usually a response to phosphate fertilizer where the ratio was lower than 0.4 or where there was a wide difference in the value of the ratio in the untreated and limed soil. The soils of the first group contain a higher percentage of colloidal material and a lower amount of iron soluble in 0.05 and 0.005 N HCl than those in the second group. Apparently the phosphorus was present in the absorbed form in the soils with a ratio of 0.9 and mainly as iron phosphate, especially at lower pH values, in the group with a ratio of 0.4.

The addition of lime to the soils in the tumbler experiment brought the reaction of all soils to approximately the same pH as shown in table 8. The pH varied from 7.92 to 8.26. The addition of aluminum sulfate reduced the pH of all the soils considerably.

The addition of lime increased the solubility of phosphorus, especially in the acid soils and the aluminum sulfate brought about a decrease in the

TABLE 7. *Phosphorus, calcium and iron soluble in 0.005 N HCl*

Soil No.	Treatment	P in p.p.m.	Ca in p.p.m.	Fe in p.p.m.	Ratio of 0.005 N HCl
					0.05 N HCl soluble P
1	O	9.52	2459	132.5	0.44
2	L	10.35	3689	48.7	0.42
3	LRP	260.00	5738	56.3	1.09
4	O	3.72	2459	64.3	0.28
5	L	5.28	2869	23.7	0.38
6	LRP	146.00	3279	26.5	0.70
7	O	10.50	2459	24.3	0.71
9	L	14.90	3279	34.6	1.01
10	LRP	203.10	2459	36.0	1.02
11	O	175.60	5328	24.0	0.95
13	L	156.60	4508	20.5	1.15
14	LRP	395.60	5328	24.0	1.44
15	O	184.20	6558	32.7	1.27
16	RP	256.30	13116	20.5	0.82
17	O	72.80	3279	90.1	0.79
19	L	52.10	4508	90.1	0.81
20	LRP	197.40	4508	160.1	1.33
21	O	8.55	2049	180.2	0.52
23	L	30.50	3279	81.9	0.65
24	LRP	125.80	3279	150.1	1.21
25	O	7.41	2869	450.5	0.17
26	L	7.97	2459	318.0	0.27
27	LRP	106.80	3279	386.1	0.69
28	O	4.78	2459	257.4	0.43
29	L	3.55	3689	84.4	0.24
30	LRP	93.41	4508	337.8	0.81

TABLE 8. *The pH and phosphorus soluble in 0.002 N H₂SO₄ in tumbler experiment*

Soil No.	Treatment	Check		Soil + 4 times lime requirement		Soil + 1% Al ₂ (SO ₄) ₃	
		pH	p.p.m. P	pH	p.p.m. P	pH	p.p.m. P
1	O	5.23	18.3	8.00	22.4	4.09	14.5
2	L	7.27	29.5	8.13	26.2	5.58	19.6
3	LRP	7.70	241.4	8.11	213.0	5.93	261.7
4	O	5.79	15.9	7.97	21.7	3.93	16.0
5	L	7.25	30.7	8.22	26.9	4.77	15.0
6	LRP	7.64	114.3	8.25	157.4	5.02	183.1
7	O	5.18	29.1	7.98	28.7	3.92	24.6
9	L	5.89	22.4	8.04	339.0	4.35	18.3
10	LRP	6.14	164.7	8.14	152.2	4.35	188.0
11	O	5.96	99.8	8.00	80.0	4.78	95.9
13	L	6.69	89.8	8.06	56.6	5.47	82.4
14	LRP	6.14	291.1	7.97	217.2	4.95	258.9
15	O	8.18	78.7	8.16*	81.1	6.58	80.1
16	RP	8.29	83.2	8.16*	50.1	7.26	85.2
17	O	6.01	54.1	8.00	39.3	4.35	44.5
19	L	7.62	47.8	8.03	41.5	4.57	27.2
20	LRP	7.87	120.9	8.05	137.1	4.82	151.1
21	O	5.12	20.8	7.92	33.8	4.19	22.5
23	L	6.53	67.5	8.02	89.8	4.61	30.0
24	LRP	5.72	107.2	7.99	153.0	4.39	133.4
25	O	5.92	44.2	8.26	28.6	4.34	14.7
26	L	7.90	44.8	7.99	33.7	5.15	20.0
27	LRP	7.92	113.4	8.21	169.9	4.93	160.1
28	O	6.05	20.3	8.10	27.3	4.40	13.7
29	L	7.88	30.1	8.05	28.4	4.43	23.7
30	LRP	7.86	134.4	8.12	172.0	5.80	164.9

* No lime added.

phosphorus soluble in 0.002 N sulfuric acid, except in the soils which had been treated with rock phosphate in the field experiments.

DISCUSSION OF RESULTS

Certain representative biological and chemical methods proposed for the determination of the available phosphorus in soils were employed in these experiments and serve as a test of some of the assumptions upon which most methods are based. It has been quite generally assumed by the proponents of the biological methods that the phosphorus available to soil microorganisms is also available to the higher plants. The assumption may be correct but it does not necessarily follow that the growth of the microorganisms is proportional to the amount of phosphorus they may obtain. There is evidence that this may not be the case (15) (18). However, if it were the case, there seems little evidence that all the phosphorus available to *A. niger* is available to all plants. The response to fertilization was different with oats and clover than with corn. It may be pointed out further that there are also a number of factors other than phosphorus supply which govern the growth of these microorganisms and some of these factors are beyond control. The biological methods for diagnosing the

phosphorus needs of soils are of limited value and cannot be recommended for general use at present.

It is well known that the concentration of phosphorus in the soil solution is low. Teakle (17) observed that it was usually within the limits of 0.05 to 10 p.p.m. It is evident that a reserve supply of phosphorus which passes into solution readily is necessary to maintain a constant supply of the phosphate ions in the soil solution and that the rate of solution and ionization is important. Some plants require more phosphorus than others and it is conceivable that the rate of solution of phosphorus in a given soil might be adequate for some plants and inadequate for others. Therefore, the concept of availability which is often incorrectly used as synonymous with solubility should take into consideration the rate of solution of the phosphate and the plant requirements.

The rate of solution of soil phosphorus is dependent upon a number of factors but chiefly upon the form in which the phosphorus occurs. The forms of phosphorus in the soil are complex but for a given soil largely determined by the reaction of that soil. Until quite recently it was commonly held that the phosphorus in the soil existed in the form of iron, aluminum, calcium, and magnesium phosphates, but the work of Mattson (6), Mattson and Pugh (7), Scarseth (14), and Ravikovitch (12) shows that much of the soil phosphorus is absorbed in the organic and inorganic colloidal complexes. The dissociation of the phosphate depends to a considerable extent upon the relative proportion of the various constituents making up these complex systems.

The observation that the amount of phosphorus dissolved by the carbonic acid solution was correlated with the pH of the soil seems to be significant. Numerous experiments have shown that liming acid soils generally results in an increased availability of phosphorus, and a rather close correlation between pH and availability of phosphorus might be expected.

Solubility under certain conditions is undoubtedly closely related to availability and there is some basis for using a 0.002 N sulfuric acid or a 1 per cent citric acid solution as the solvent. Since the phosphorus may exist in the soil in a number of forms and these forms may vary in the same soil under different conditions, it is not likely that a simple method based on solubility will ever solve the problem. The approach of von Wrangell (22) and more recently the work of McGeorge (8) in determining the rate of solubility of soil phosphorus seems most logical. Also, the work of Fisher and Thomas (3) in determining the forms of phosphorus in the soil promises much help in the solution of the problem of the selection of a method for determining available phosphorus in soils.

SUMMARY

Thirty soils from nine cooperative fields of known crop response to phosphate fertilizers were sampled and the "available" phosphorus determined by four biological and five chemical methods. No one method employed indicated accurately the phosphate needs of all the soils tested. The different crops responded differently to the application of a phosphate fertilizer. The solubility of phosphorus in carbonic acid at 25° C. and 50 mm. of water pressure appeared to be related to the pH of the acid soils. In the heavier soils where the phosphorus apparently was in the

absorbed or exchangeable form the Bray test was fairly indicative of the phosphorus needs of the soil. In general these results indicate a close relationship of phosphorus availability to the form of phosphorus in the soil and its rate of solution. It also appears that the plant needs for phosphorus must likewise be considered.

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PLATE I

Apparatus for shaking soils in water saturated with carbon dioxide.

PLATE I



MIGRATION OF SHORE BIRDS AT GOOSE LAKE, HAMILTON COUNTY, IOWA DURING THE FALL OF 1936

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With the presentation of this paper the writers hope to make an additional contribution to shore bird investigations in Iowa. The data upon which this contribution is based were collected at Goose Lake, Hamilton County, Iowa, from August 21 to November 8, 1936. Correlative observations from other localities are included in the discussion of individual birds.

Goose Lake, typical of the Wisconsin Drift marshes, is located one-half mile east of Jewell, Iowa. In respect to the mass distribution of the marshes and lakes of northern Iowa, Goose Lake, being one of the most southern, appears near the apex of the group. It is about 80 acres in area. During the period of observation not more than 20 acres of the marsh were in open water, and the remainder was grown up to a *Scirpus-Typha* (bul-rush-cat-tail) associates. Three hundred yards of open shore line on the east margin of the marsh provided the waders with beaches and mud-flats. This shore line was apparently maintained by the watering and feeding of cattle, domestic ducks and domestic geese. The Wilson Snipe (*Capella delicata*) preferred three acres of wet meadow adjoining the open shore line on the south and two acres of similar range around the northeast margin of the marsh.

Some preliminary shore bird observations were made on August 21 and 25. These were followed by accurate counts at two-day intervals from September 2 to November 8. The included flight graphs are based upon these counts.¹ One writer checked on the other in the field, and necessary verifications were made by reference to specimens collected in northwest Iowa and deposited in the Iowa State College Museum by L. J. Bennett and Gerald B. Spawn. No specimens were collected during the course of these investigations.

Twenty-three species of birds are discussed here in sequence according to their order in the American Ornithologist's Union check-list (1931).

SEMIPALMATED PLOVER

Charadrius semipalmatus Bonaparte

One of these birds was observed on September 22. It is considered a fairly common spring and fall migrant. Conclusive evidence of this has been presented for northwest Iowa by Bennett (1934) and Spawn (1935).

KILLDEER

Oxyechus vociferus vociferus (Linnaeus)

A common migrant and summer resident. A total of 613 birds were observed from August 21 to October 30. Counts were taken only in the

¹Thanks are due Dr. A. E. Brandt of the Department of Mathematics at Iowa State College for advice in connection with the preparation of the graphs.

immediate vicinity. The peak of migration occurred between September 8 and 14 (fig. 1).

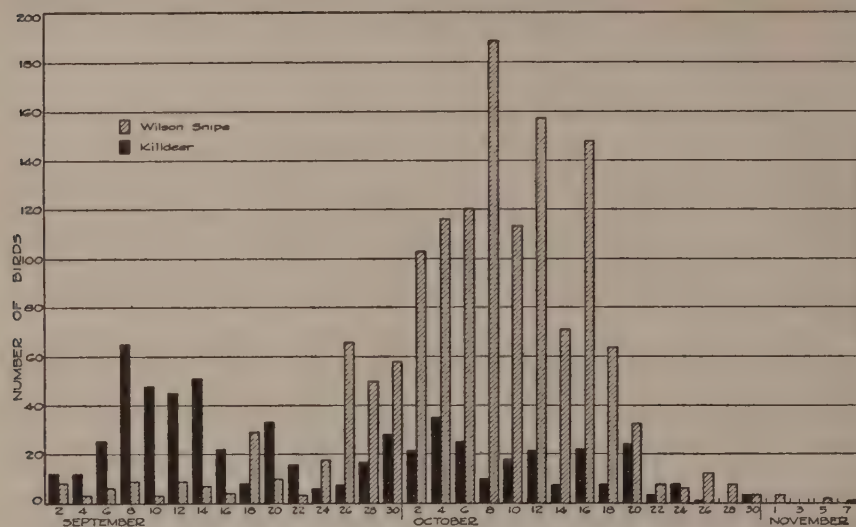


Fig. 1. Fall (1936) flight of the Wilson Snipe and Killdeer at Goose Lake, Hamilton County, Iowa.

AMERICAN GOLDEN PLOVER

Pluvialis dominica dominica (Müller)

This bird is considered an uncommon migrant. Single birds were observed on September 20 and November 8. The marsh was frozen over and several inches of snow were on the ground when the last observation was made.

Twenty of these birds spent the day in and around a closely-grazed meadow about nine miles directly north of Ames on October 24. The birds were flushed repeatedly, but they continued to return to the meadow. At dusk they took to the air of their own choosing, and after circling the area several times they disappeared from sight.

BLACK-BELLIED PLOVER

Squatarola squatarola (Linnaeus)

This bird is generally listed as a rare migrant. A single bird was recorded during the fall of 1934 on Lost Island Lake, Clay County (Spawn, 1935). Eleven birds were observed at Goose Lake from September 18 to October 26. On October 22 a flock of five was seen wading about in the shallow water along the east margin of the marsh. They appeared to be picking up food from the surface of the water. Later they were flushed from the short grass of the meadow adjoining the east shore.

RUDDY TURNSTONE

Arenaria interpres morinella (Linnaeus)

A lone bird of this rare species was observed on four consecutive dates from September 4 to 10.

AMERICAN WOODCOCK

Philohela minor (Gmelin)

On July 20, Dr. H. M. Harris observed three birds in the wooded preserve north of the Iowa State College campus at Ames. Scott observed a single bird in the same locality on July 21 and 23.

WILSON'S SNIPE

Capella delicata (Ord)

An aggregate of 1,452 of these birds was observed from August 21 to November 8. A single bird was found hiding in the sedges on November 8 after the marsh had frozen over. A count of 189 birds was made on October 8 on five acres of wet meadow (fig. 1). This moderately grazed meadow was preferred to areas supporting rank growths of bulrushes and cat-tails. The meadow vegetation was from 6 to 18 inches tall and stooled throughout. The hoof prints of stock about the meadow provided probing areas. A suitable balance of food and shelter seemed to have been provided.

Mrs. Harold Peasley and Scott observed a single bird of this species at a flowing spring along Beaver Creek near Johnson Station in Polk County on January 4, 1937.

SPOTTED SANDPIPER

Actitis macularia (Linnaeus)

Although this bird is a common migrant and summer resident throughout the state, only three birds were observed at Goose Lake. The dates for these records are: August 25, September 2 and September 6.

EASTERN SOLITARY SANDPIPER

Tringa solitaria solitaria Wilson

A fairly common migrant throughout the state. A single bird was noted on September 6. The mud flats of the Des Moines River may have proved more inviting to this bird along with others such as the Semipalmated Sandpiper, Semipalmated Plover, Spotted Sandpiper and White-rumped Sandpiper.

GREATER YELLOW-LEGS

Totanus melanoleucus (Gmelin)

Although not so numerous as the Lesser Yellow-legs, this bird is listed as a fairly common spring and fall migrant (DuMont, 1934). Fifteen of

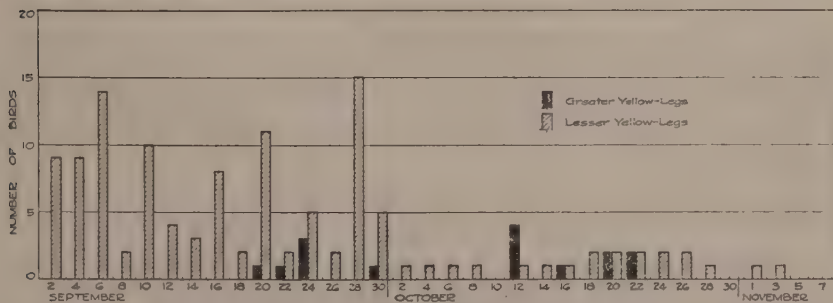


Fig. 2. Fall (1936) flight of the Greater Yellow-legs and the Lesser Yellow-legs at Goose Lake, Hamilton County, Iowa.

these birds were observed from September 20 to October 22 (fig. 2). The feeding of this bird proved quite comical. On several occasions it was observed running in small circles with the bill dipped in the water and the long legs kicking out behind as if in pursuit of some aquatic insect.

LESSER YELLOW-LEGS

Totanus flavipes (Gmelin)

A total of 146 of these birds was seen during the period of observation (fig. 2). One crippled bird remained in the area from October 2 to November 3 as the only representative of the species during that time except for a single bird which accompanied it from October 18 to 26. On September 28, an exceptionally cold day, the writers encountered 15 unusual appearing shore birds. The birds proved to be Lesser Yellow-legs with their necks pulled in, bodies slunk down and feathers fluffed out in defense against the cold.

PECTORAL SANDPIPER

Pisobia melanotos (Vieillot)

A widely distributed transient. The peak of migration for this species probably occurred before observations were begun. A total of 28 birds was counted. A flight of 12 was seen on August 28.

WHITE-RUMPED SANDPIPER

Pisobia fuscicollis (Vieillot)

"A fairly common migrant in the spring and fall" (DuMont, 1934). Only one individual of this species was observed. It was observed in company with five Red-backed Sandpipers on October 24. Spawn records a single bird for Lost Island Lake during the fall migration of 1934.

BAIRD'S SANDPIPER

Pisobia bairdi (Coues)

A single bird was observed on September 22 in company with a Least Sandpiper. This fairly common migrant is not recorded as frequently in the fall as in the spring.

LEAST SANDPIPER

Pisobia minutilla (Vieillot)

A total of 40 birds was counted from August 25 to October 20. The flight was rather continuous from August 25 to October 4, with not more than four birds being observed during that time on any single occasion. No birds were seen until three were recorded on October 20. They were almost always in company with individuals or small numbers of other short-legged sandpipers. They invariably fed in shallow water along the eastern shore line.

RED-BACKED SANDPIPER

Pelidna alpina sakhalina (Vieillot)

There appeared to be a definite migration of these birds from October 8 to October 30 (fig. 3). Forty-nine birds were observed during that time. These friendly little sandpipers with the down-curved bills were quite gregarious in their feeding. The little flocks worked swiftly and efficiently

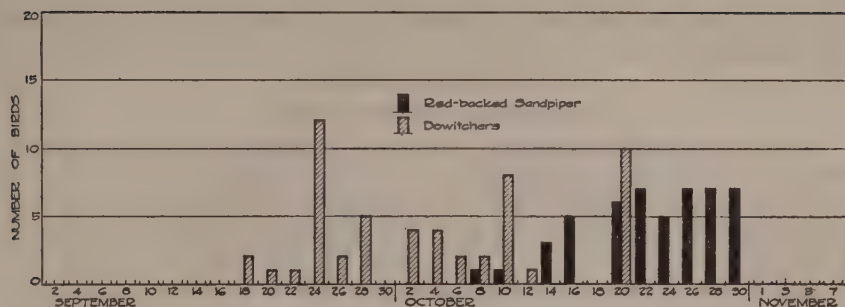


Fig. 3. Fall (1936) flight of the Red-backed Sandpiper and Dowitchers at Goose Lake, Hamilton County, Iowa.

through the shallow water of the beach, and occasionally they were seen feeding "breast deep."

EASTERN DOWITCHER

Limnodromus griseus griseus (Gmelin)

and

LONG-BILLED DOWITCHER

Limnodromus griseus scolopaceus (Say)

The impossibility of properly separating the Eastern Dowitcher and the Long-billed Dowitcher in the field makes discussion as one species necessary. Fifty-four of these birds were observed from September 18 to October 20 (fig. 3). Almost invariably the compact groups of these birds were seen feeding in shallow water along the sandy-loam beach.

STILT SANDPIPER

Micropalama himantopus (Bonaparte)

Thirty-four of these sandpipers were observed during a continuous flight from September 18 to 28. They are considered as fairly rare migrants by DuMont (1934). Eight of these sandpipers, observed on September 28, all puffed up in defense against the cold, presented an odd appearance.

SEMPALMATED SANDPIPER

Ereunetes pusillus (Linnaeus)

A common spring and fall migrant. Eight birds were seen from September 18 to October 8. Three of these birds were recorded on October 6.

WESTERN SANDPIPER

Ereunetes mauri Cabanis

The status of this bird is undetermined (DuMont, 1934). It is reported as a common migrant by Bennett (1934) and Spawn (1935). An individual was observed on September 30.

SANDERLING

Crocethia alba (Pallas)

This rare migrant was represented by a single bird seen on September 18. It was feeding in the shallow water of the sandy-loam beach in com-

pany with two Semipalmated Sandpipers, one Least Sandpiper and two Stilt Sandpipers.

WILSON'S PHALAROPE

Steganopus tricolor Vieillot

"A fairly common migrant. Formerly a common summer resident, breeding in the northern part of the state" (DuMont, 1934). Wilson Phalaropes, which appeared to be nesting, have been reported for Dewey's Pasture and Barringer's Slough in Clay County, Iowa, by Bennett (1936). An individual was observed at Goose Lake on August 25.

NORTHERN PHALAROPE

Lobipes lobatus (Linnaeus)

"An uncommon migrant along the Missouri River Valley, rare in other parts of the state" (DuMont, 1934). On October 16, a single bird was observed from a boat about 30 yards from the east shore line. The bird was perched upon a lily pad and did not fly away until approached quite closely.

SUMMARY

1. The heavy migration of the Wilson Snipe was over at Goose Lake by October 22, 1936.
2. The Wilson Snipe exhibited a preference for wet meadows which had been moderately grazed.
3. The ratio of Greater Yellow-legs to Lesser Yellow-legs was approximately 1:10 at Goose Lake during the fall of 1936.
4. The Spotted Sandpiper, Eastern Solitary Sandpiper, Semipalmated Plover, White-rumped Sandpiper and Semipalmated Sandpiper, all common migrants throughout the state, were rare at Goose Lake during the fall of 1936.
5. Twenty-two Golden Plovers and eleven Black-bellied Plovers were seen in central Iowa during the fall of 1936.

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TWO METHODS FOR MEASURING EGESTION TIME FROM LARGE INSECTS

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Various standard equipments are available for collecting fecal pellets from smaller experimental vertebrate animals such as guinea pigs, rats, and mice, but all demand apparatus which is too cumbersome for, or not adaptable to, use with even large insects. To collect data on egestion time from insects such as the American roach, several of the larger grasshoppers, and older instars of silkworm and tobacco worms, two sets of apparatus have been devised of materials found, for the most part, in almost all zoological and physiological laboratories. Both methods consist, as do others built for small mammals, in the use of cages to keep test animals, and a calibrated moving surface for collecting dropped fecal pellets.

Individual cages (fig. 1) used for insects consist of a tube of copper screen-wire, 5 inches long, $1\frac{1}{4}$ inches in diameter, and held together by several globules of spot solder. About $\frac{1}{8}$ inch of the ends of the tube is folded over to form a "hem" so that corks may easily slip in and out of the tube. The upper end of the cylindrical container is closed with a cork through which extends a "J"-shaped watering tube, the lower end of which is flared to form a cup about $\frac{7}{16}$ inch in diameter at its widest part. The lower end of the screen tube is left open and fits into a rack (fig. 2, F) whose lower surface is covered with $\frac{1}{4}$ -inch mesh galvanized iron screen. Cages are held vertically in the rack by a second board through which the upper ends of the containers protrude. The two boards with the cages are held in position by clamps on ringstands (fig. 2, C). Below the cages is the platform for collecting egested pellets (fig. 2, H).

Two types of moving platforms have been used. One employs revolving circular disks of card-board or pressed wall-board fastened to the vertical axle of an ordinary clockworks kymograph (fig. 2, J). The kymograph drum is left on the axle to give additional support to the disk. Speed of the clockworks can easily be regulated so that one revolution of the disk occurs in about 16 hours. The surface of the moving disk is marked off in hours and minutes by radii of the circular area. The calibrated surface may be covered with cellophane which can be kept clean by wiping with a damp cloth. Disks of various diameters are used, depending on the laboratory space available. Smaller ones—about 2 feet in diameter—are made of card-board; the largest—4 feet in

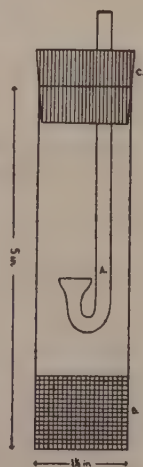


Fig. 1. Diagram of special cage used for egestion time studies. A = "J"-shaped watering tube; B = copper screen-wire; C = cork at upper end of cage through which watering tube extends.

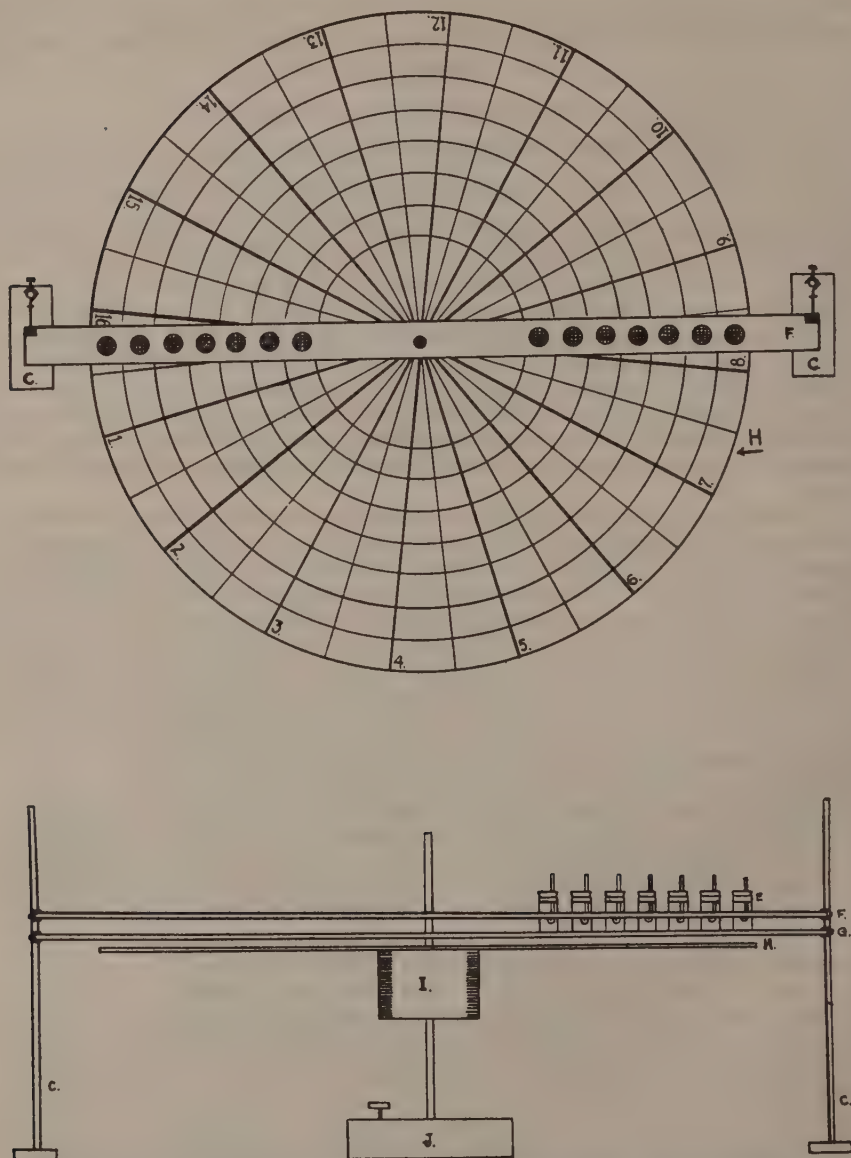


Fig. 2. Top and side views of disk type of apparatus for collecting fecal pellets. C=ringstand; E=cages; F and G=rack for holding cages, G with holes covered with $\frac{1}{4}$ -inch mesh; H=pressed wall-board disk with calibration radii; I=kymograph drum to support disk; J=kymograph clockworks.

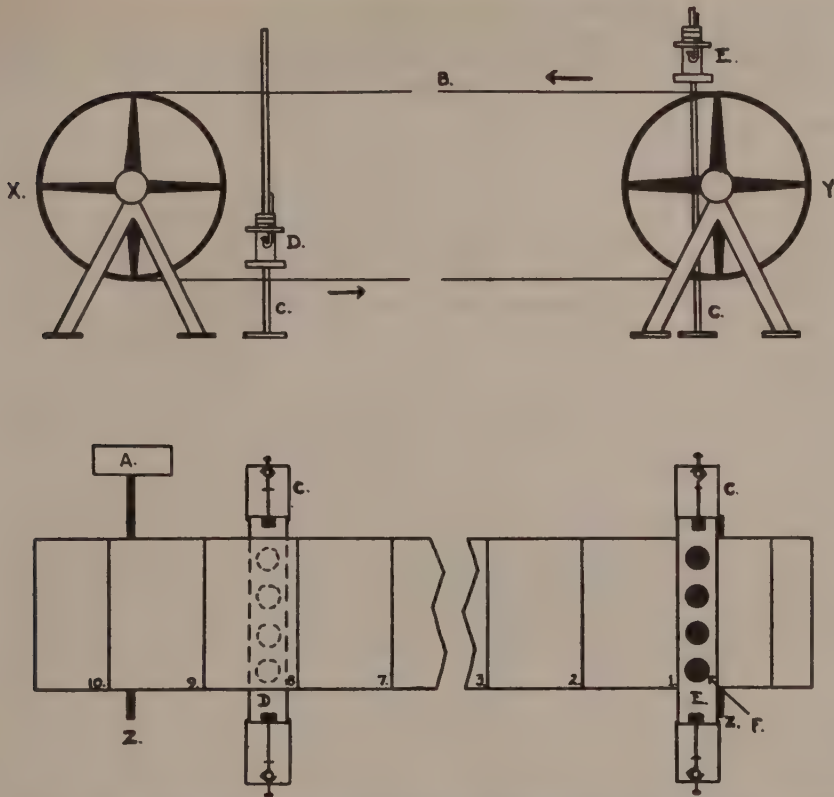


Fig. 3. Side and top views of belt type of apparatus for collecting fecal pellets. A = "Telechron" motor; B = continuous belt of kymograph paper; C = ringstands; D = lower row of cages; E = upper row of cages; F = $\frac{1}{4}$ -inch mesh covering holes in lower part of rack; X = motor driven cylinder; Y = freely moving cylinder; Z = axles of cylinder.

diameter—is made of wall-board covered with white paper on which calibration radii were marked.

The second type of collecting device consists of a continuous belt of glazed kymograph paper, 8 inches wide, supported by two kymograph cylinders (X and Y, fig. 3) on horizontal axles. One of the drums is disengaged from the kymograph driving wheel and rides freely in its original bearings; the other is powered with a "Telechron" electric-clock motor (fig. 3, A) regulated so that the revolving drums make one revolution in 2 hours. The paper belt is calibrated with cross lines for hour, $\frac{1}{2}$ hour, and $\frac{1}{4}$ hour intervals. The moving belt of paper can be of various lengths. The longest attempted so far is 20 feet, which allows about $9\frac{1}{2}$ feet of recording surface before the belt turns to make the lower part of its

circuit. When long belts are used, they must be supported at points along their length by cross bars of some type to take care of the sagging which cannot be avoided. Sections of glass tubing supported by clamps on ring-stands serve very well for this purpose.

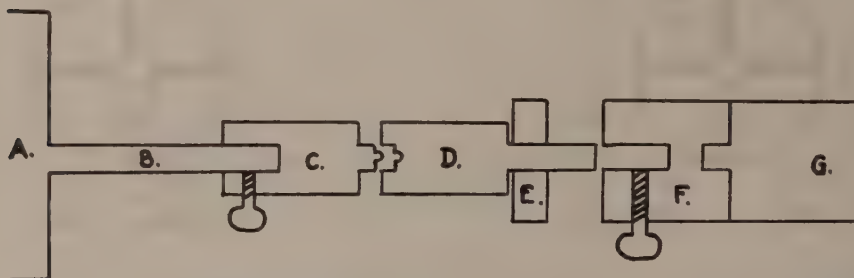


Fig. 4. Drive-shaft for moving axle of cylinder X in fig. 3. A = motor; B = driving axle of motor; C and D = sections of drive shaft which can be separated whenever necessary; E = bearing; F = "cap" fitting on D; G = axle of cylinder X.

To turn the axle (fig. 2, Z) of kymograph X with the electric motor, A, several attachments were necessary to make a drive-shaft which could be easily separated. A metal "cap" (fig. 4, C) with cross-ridge and centering pin is fastened to the axle, B, of the motor, A, by a set-screw. The ridge and pin fit into a slot and central depression at the end of a section, D, which passes through a bearing, E, screwed into the original opening of the kymograph stand. A second "cap," F, is held with set-screw on the kymograph end of D. This second "cap," F, is fitted into the kymograph axle, G, in the same ridge and slot manner found between C and D. When not in operation, C and D are pulled apart and the paper belt can be rotated without stripping the set of gears which reduce the speed of the shaft from the motor. (These speed-reducing gears are a part of the motor as purchased from the manufacturer.)

In preparation for the tests, the insects are starved for a short time (the length of time depending on the species), then placed in individual vials and, for one hour, are allowed access to food which has been mixed or covered with a colored material such as carmine, congo red, or ultramarine blue. Time of first ingestion is noted. After feeding, specimens are placed in individual cages above the pellet collecting mechanism which is then set in motion. Records are kept of times when egestion occurs. Dropped pellets are broken up in a mortar and examined microscopically for presence of dye. The time elapsing between first ingestion of dye and first appearance of the colored substance in the feces is designated as "egestion time."

DISCUSSION

Methods described above for measuring egestion time have been especially successful with the American cockroach, *Periplaneta americana*. Dimensions of the cage described have been selected particularly for this insect. The screen containers are long enough so that the internal space is not too confining even for adult specimens. It is high enough so that the insect's antennae need not be twisted or bent; it is large enough so

that a contained individual can easily move about on the screen-wire which gives a suitable foothold. The diameter, however, is such that the animal does not easily fit across the bottom or top of the cage. This situation forces the specimen to take a position usually parallel to the long axis of the cage. Since many insects prefer to keep the anterior part of the body upward when on a vertical surface, this is an advantage in the arrangement because fecal pellets can then drop downward through the bottom mesh. Occurrences of ecdysis of nymphs and oviposition of adult females seem evidence that the confinement is not particularly disturbing. For other insects, obvious modifications in cages are necessary. Smaller insects will demand smaller unit dimensions of the mesh at the bottom of the tube of screen-wire. For many leaf eating larvae, the water tube may be omitted.

Distance between cages on the supporting racks can be varied, but about $\frac{3}{4}$ inch, which allows enough space between containers for handling and working, has been found satisfactory.

Carrying capacity of the apparatus may be increased at least two ways. First, if food colored with only one dye is fed, the data on the collecting surfaces may be checked at intervals of about 8 hours so that no part of the collecting surface goes under more than one set of cages before observations are made. Second, if one set of animals is fed food with one dye, and a second set fed food with a different dye, double racks of containers may be used. Microscopic examination of the broken pellets will show which individual is responsible for a pellet located at a certain point on the collecting device, should rack arrangements be such that two animals have passed over the same section of the calibrations.

SUMMARY

1. Two methods are described for making measurements of egestion time from large insects. Insects are confined in special cages above a moving calibrated surface which consists either of a revolving disk turned by kymograph clockworks, or a continuous belt of paper supported by two horizontal cylinders, one of which is powered by a geared-down electric motor.

2. Preparatory methods of feeding test animals are given.

3. Suggestions are listed for varying the cages for different species of insects, and for increasing the carrying capacity of the mechanisms.

4. Four figures showing details of construction are included.

TEMPERATURE PREFERENCE OF THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD). (THYSANURA)

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The firebrat, *Thermobia domestica* (Packard), as its common and scientific names imply, prefers remarkably high temperatures. Oudemans (1889) stated that in Amsterdam these insects were termed "Ovenvogeltjes" (little oven-birds) because of their occurrence about the ovens of bakeries. Oudemans found that his captured specimens died at room temperatures even in summer; but he was able to induce others to live and molt in an incubator at 30° Centigrade. Spencer (1930) stated that firebrats require temperatures between 90° and 110° Fahrenheit (equivalent to about 32° to 43° C.) in order to thrive. The writer (Adams 1933) began to culture firebrats at high constant temperatures, around 37° C. The cultures begun in 1931 continue to thrive and increase at the time of writing in 1937.

In order to determine the temperature preferences of these insects more accurately the writer constructed a thermal-gradient apparatus to test their thermotropism. The preliminary results confirmed Spencer's statement as to the range of the preferred temperatures and indicated that within this range the animals much prefer temperatures between 36° and 39° C. A description of the apparatus was placed in the author's doctoral thesis (1935) of which the abstract is to be published (Adams 1937). The investigation was resumed in 1936, the apparatus was improved and new data were obtained. The results of these more recent studies are presented here together with a description of the apparatus, which, with modifications, might be adapted to the investigation of the thermotropism¹ of small animals of many kinds.

A THERMOTROPOMETER DESIGNED FOR THE FIREBRAT

The final form of the apparatus is illustrated in Fig. 1. The principal part is a tin-plated sheet-metal trough 48 inches in length, 5 inches in height and 8 inches in width. Vertical sliding partitions made of the same material divide the interior into twelve chambers. The partitions project slightly above the rim of the trough. Each chamber has a glass lid and a fibre-board cover. A thin asbestos mat fitted into the bottom of the trough serves as floor covering for the chambers. Slightly above the floor of each chamber is a small circular opening through which a thermometer is inserted so that the bulb rests near the center of the floor of the chamber. Supported well above the thermometer in each chamber is a shallow dish, about three and one-half inches in diameter, for the humidity-controlling salt solution. A glass vial about one-quarter inch in diameter, filled with water and plugged with a small cotton wick, lies in each chamber to provide additional moisture for ovipositing females. A wad of ab-

¹ The term thermotropism is used here to designate movement in response to an external heat stimulus.

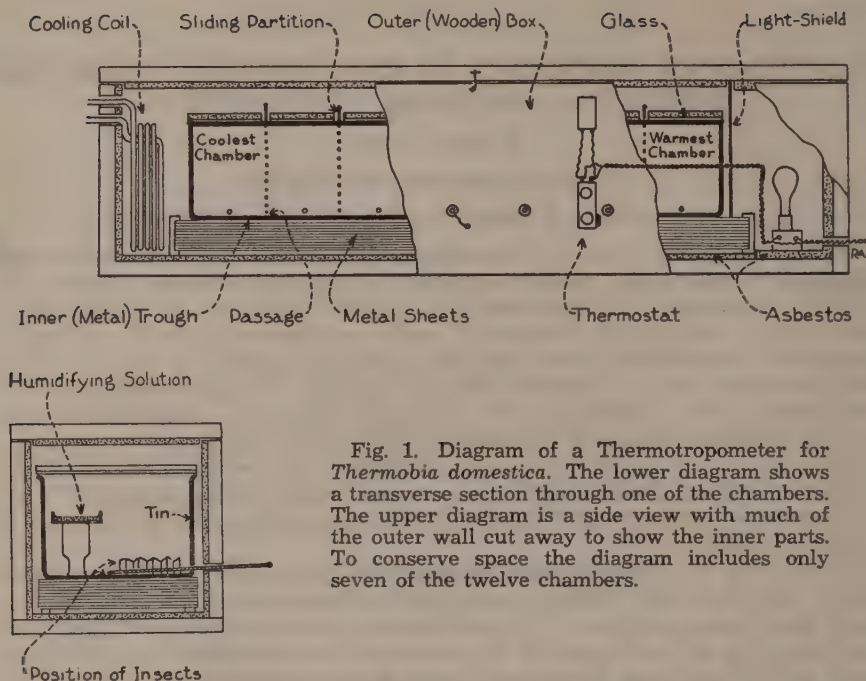


Fig. 1. Diagram of a Thermotropometer for *Thermobia domestica*. The lower diagram shows a transverse section through one of the chambers. The upper diagram is a side view with much of the outer wall cut away to show the inner parts. To conserve space the diagram includes only seven of the twelve chambers.

sorbent cotton about half-an-inch in diameter is added for the reception of eggs. Without these latter provisions mature females are likely to be restless and exhibit wanderings not traceable to the temperature factor. Food for the insects, oat-flour and dried milk, is sprinkled on the floor of the chamber and two strips of paper about one inch in width and six inches in length are plaited transversely and stood on edge as resting places. The sliding partitions between the chambers come to rest upon nail-like bits of wire each of which is manipulated from the outside of the apparatus by a slender copper wire which passes through the same opening as does the thermometer. These removable supports are of such thickness that when they are in place there is a slit-like opening between the lower edge of the partition and the asbestos mat just large enough to allow the firebrats to pass under the partition freely. When the supports are withdrawn the partitions drop down of their own weight.

The trough rests upon twenty sheets of galvanized iron two inches wider and several inches longer than the trough. The metal sheets are clamped together as a heat-distributing unit. The whole is encased in a wooden box with one-inch walls. The box is lined with asbestos and fibre-board insulation. An electric light bulb for heating the chambers is mounted within the wooden box at one end. A DeKhotinsky thermostat is mounted on the outside of the box with its bimetallic spiral projecting through the walls of the box and the trough into the second chamber. The thermostat is set in the circuit to the lamp. The light rays of the lamp are excluded from the trough by a metal shield. Within the box at the oppo-

site end of the trough is a coiled copper tubing through which water may be circulated to cool that end of the apparatus. This feature was seldom needed in practice. The hinged lid of the box is fitted to exclude light.

The apparatus is designed to provide living conditions for the firebrats over periods of weeks. To prevent distortion of the results by overcrowding of the central chambers the number of insects used is limited to about fifty adults. The partitions are set upon their metal supports so that the insects may be free to move throughout the length of the gradient of temperatures. Firebrats are characteristically unable to climb upon the smooth metal walls but must remain upon the asbestos mat and the plaited paper strips close to the bulbs of the thermometers. At intervals of four or more hours the distribution of the insects in response to temperature is obtained in this way: The supports of the sliding partitions are quickly withdrawn and the insects are thus locked in the chambers. The thermometers are read and the apparatus is then opened and the number of individuals in each chamber counted at leisure.

When, in a series of chambers, a gradient of temperatures is set up there will be a tendency for an opposite gradient of relative humidities to develop. This tendency is here largely offset by the use of saturated salt solutions containing an excess of the salt. For this purpose potassium chloride solutions are placed in each chamber. Since the apparatus is not air-tight and the air in the chambers is nearly static the relative humidities theoretically obtainable with this salt at these temperatures are not obtained. The purpose of the salt solution is, however, served when the relative humidities in the various chambers at various temperatures are brought within a few per cent of equality. Each time the apparatus is examined to record the positions of the insects the dew-point of the air in one of the chambers is measured. This instrument necessitates for the chamber a special cover partly of wood, partly of glass, with a small opening for the insertion of the instruments. As Sweetman (1933) has pointed out, it takes considerable practice to get consistent results with this method. The values obtained in the various chambers at various readings ranged between 67 and 73 per cent R. H. It may be that the actual percentages stated are subject to a more or less constant error (they are probably too low); it is their comparative uniformity which is important. Having considerable experience in the rearing of firebrats the writer believes that throughout the various chambers the insects are almost uniformly comfortable with respect to their humidity relations. When firebrats are kept in air of lower relative humidity than about 60 per cent they become attracted to the moist cotton wicks of watering vials to which they will cling for hours. Although each chamber contained such a watering vial the phenomenon was seldom seen in these experiments. That the firebrats in the thermotropometer were under generally favorable conditions is further confirmed by the reproduction which occurred during the weeks of confinement. Many of the resulting nymphs reached the fourth instar before the experiments were discontinued and the insects removed.

The firebrats used were fully grown specimens of both sexes, reared in the laboratory. For the final series of experiments, reported below, the specimens were taken from an incubator operating at about 35° C. In order to keep the number of test animals at 50 those which escaped, or were killed by the sliding partitions, were replaced by others from the laboratory stock.

RESULTS

Over 100 trials of the apparatus were made. Sixty of these were run before the apparatus and methods approximated the above description. In most of the early tests sodium chloride was the salt used for humidity control and the humidities obtained were not measured. For these reasons the data from the earlier tests are regarded as preliminary. In the summary of the first 34 trials the frequency distribution of the insects along the temperature scale was such that the greatest number of insects was recorded at 39° C. and the arithmetic mean point of the distribution was 37.5° C.

The results of the more recent trials are very similar. Some examples of the records are shown in table 1. By manipulation of the thermostat the temperature was caused to fall in the second chamber over a period of days. In the other chambers this tendency was more or less offset by a rising room temperature in trials Nos. 85 to 90. During trial No. 91 the cooling coil was operated with the result that the temperature dropped throughout most of the chambers and the insects migrated toward the remaining warmer chambers.

Fig. 2 shows graphically the summary of 41 trials. About 50 insects were run in each trial so that the figures show the distribution on the temperature scale of 2009 response-positions taken by firebrats in the apparatus. Although the temperatures indicated by the thermometers inserted into the chambers were sometimes read to half-degrees the records are here condensed to the whole-degree scale. Each sum for a half-degree

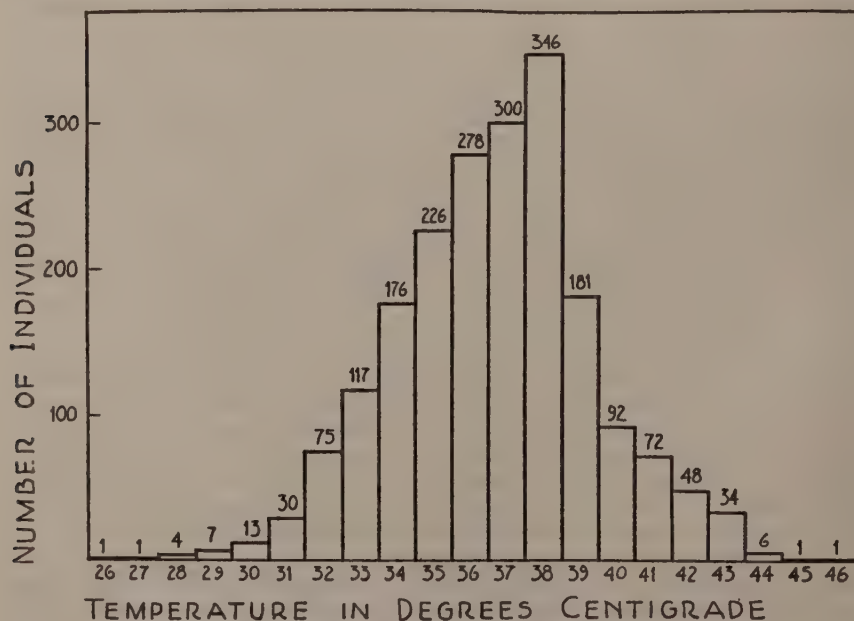


Fig. 2. Frequency distribution of 2009 positions taken by firebrats in response to temperatures in a thermotropometer. Each number written above a vertical bar is the sum of the number of insects recorded at one temperature in the apparatus.

TABLE 1. *Temperatures, humidities and self-distributions of Firebrats in eight consecutive trials in a thermotropometer*

Trial No.	Hours Since Last Reading	Individual Chambers of the Apparatus										
			XI	X	IX	VIII	VII	VI	V	IV	III	II
85	7	Tempr.	29.5	30	31	32	33.5	35	37.5	41.5	46.5	54
		Rel. Hum.	70%
		No. Insects	0	0	0	3	2	10	33	3	0	0
86	17	Tempr.	29	30	30.5	31.5	33	35	37.5	41.5	46.5	54
		Rel. Hum.	73%
		No. Insects	0	0	0	3	2	18	24	4	0	0
87	5	Tempr.	30	30.5	31	32.5	34	36	38	42	47	55
		Rel. Hum.	73%
		No. Insects	0	0	0	3	2	8	35	3	0	0
88	5	Tempr.	31	32	32.5	33.5	35	36	38.5	42	46	53
		Rel. Hum.	70%
		No. Insects	1	2	2	1	4	11	27	2	1	0
89	11	Tempr.	31.5	32	33	34	35	36.5	38.5	42	46.5	53
		Rel. Hum.	72%
		No. Insects	0	2	1	2	1	18	25	2	0	0
90	6	Tempr.	32	32.5	33	34	35	36.5	39	42	46	51.5
		Rel. Hum.	72%
		No. Insects	1	2	0	3	3	16	23	3	0	0
91	17	Tempr.	26	28	29.5	31	32.5	34.5	37	40.5	44.5	51.5
		Rel. Hum.	73%
		No. Insects	0	0	0	1	2	7	33	7	0	0
92	6	Tempr.	26	28	29	31	32	34	36.5	40	44	51.5
		Rel. Hum.	70%
		No. Insects	0	0	0	0	3	5	22	20	0	0

point was split into equal (or, in the case of odd-numbered sums, nearly equal) portions, one of which was added to the sum at the whole-degree point above and one to that below. Although the modal point of the distribution is at 38° C. the arithmetic mean of the distribution is at 36.6° C.

DISCUSSION

The difference between the results in the preliminary and in the final trials is roughly a matter of one degree in temperature. The arithmetic mean of the former is 37.5° C. and of the latter 36.6° C. Much of this difference is probably due to a difference in the positions of the thermometer bulbs in the chambers in the two sets of trials. In the latter the thermometers were inserted into the chambers slightly on the cooler side of the middle of the chamber space. The error so produced is likely to have been as much as a half-degree or more in the warmer chambers. If, in view of these facts, we correct the data by an upward shift of one-half degree the arithmetic mean of the distribution moves to a point slightly above 37° C. and the point of greatest concentration becomes 38.5° C.

One of the purposes of the study was to determine the temperature most preferred by the animals. It is evident that this point should be near

the center of the thermotropic distribution. It may be debated, however, which of the types of averages is to be chosen as the best indicator of this most preferred point (at which, theoretically, the animal in the thermotropometer experiences a minimum of thermal stimuli). The definition in the earlier paper (Adams 1937) points to the mode of the distribution as the best indicator, but, since the data were limited in quantity to the results of 34 trials with 23 to 44 insects in the apparatus, the arithmetic mean, 37.5°C ., which was calculated from all the data was tentatively chosen as the best approximation of the "thermotactic optimum" then available. In view of the more recent trials, however, in which again the mode is about one and one-half degrees higher than the arithmetic mean, it seems reasonable to suspect that negative skewness is a characteristic of thermotropic distribution at biologically high temperatures; and that, provided sufficient data are utilized to derive it, the mode of the distribution should be accepted as the most preferred temperature. Since the magnitude of the smallest change in temperature required to cause a measurable change of response by the animals may be any fraction of one degree Centigrade, the precision with which the most preferred temperature may be determined remains for further investigation.

While the preferred temperature, determinable with a thermotropometer, must not be confused with the general optimum, determinable only by lengthy cultural experiments, the temperature the insect prefers is probably very close to, if not identical with, the temperature at which it thrives best. An investigator setting out to study and to rear a species of insect may save himself much labor by testing some specimens in a thermotropometer and thereby quickly gaining an approximation of the temperature at which he should begin his cultures in order to get at least fairly satisfactory results. The thermotropometer will show him not only the central tendency but also the limits of the insect's temperature preferences; and, as with the firebrat, these limits are likely to correspond roughly with the cultural limits.

The preferred and the optimum temperatures are not to be confused with the temperature at which there is maximum velocity of development. According to rearing experiments (Adams 1937) the life-cycle of the firebrat from egg to egg was completed in as few as 7 to 8 weeks at 42°C . But the mortality was higher than that at 37°C ., at which point the cycle required 11 to 12 weeks.

SUGGESTIONS REGARDING THERMOTROPOMETERS

Although the apparatus described above is simple and inexpensive it has several shortcomings. In accordance with the laws of heat the drop in temperature from the warmer to the cooler end of this apparatus is not uniform. Each succeeding chamber from the warmer to the cooler end covers a smaller portion of the temperature scale than the preceding one.

Since only one temperature is recorded from a chamber at a reading any one point of temperature which occurs in the warmer chambers is likely to appear in the records less frequently than a point of temperature which occurs in the cooler chambers. This probably accounts for the irregularities of the data for points above 36°C . A larger number of experiments might have given a smoother curve. Furthermore, the temperatures in the cooler chambers remote from the thermostat were in-

fluenced by the fluctuating temperatures of the room. Another criticism is that the chambers have no air-circulating system to insure uniformity of air conditions within their enclosed spaces.

In view of these criticisms the writer recommends that each chamber in a thermotropometer be equipped with its own units for the control of temperature, humidity and air-movement. Such an apparatus would consist essentially of a series of individually air-conditioned cabinets set side by side with narrow closable openings cut through the contacting sides to allow the animals to move freely from cabinet to cabinet. It would have the disadvantages of being rather complicated and costly to construct.

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STUDIES ON BROOD A JUNE BEETLES IN IOWA¹

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White grubs are the immature stage of a moderately large genus (*Phyllophaga* (Harris)) of beetles known as "June beetles" or "May beetles." At the present time approximately 123 species are recorded as occurring in the United States and Canada. Various investigators in different localities report that from 10 to 30 or more species may be collected in almost any region with the exception of that area immediately along the west coast. In Iowa and other midwestern states, the larvae are considered one of the more serious pests of field and garden crops. Very often, too, the adults are destructive as they are foliage feeders and defoliate trees and other plants in certain localities. The present paper is the result of a survey of Brood A adults and brings together all the known records of this brood for the state, as well as certain field studies on stomach poisons which were carried out to parallel a series of laboratory experiments on similar materials and published in an earlier paper by Andre and Pratt (1936).

BROODS

Numerous papers are recorded in entomological literature dealing with the life history and habits of the various members of this genus. It has been determined that the length of life cycle of the different species varies from one to four years, depending on the particular species involved, climatic conditions, and the locality where they occur. In Iowa, however, it may be said that usually three years are required to complete the development from the time the egg is laid until the adult emerges to feed. As a brood appears each year they are called Broods A, B and C. During a Brood A flight year (1932 and 1935) the adults are extremely abundant. As 1935 was the year for Brood A adults to be present, it was decided that a survey should be made to determine the number of species present, the distribution of the various species, and the food plants in the different localities. It is planned that this same type of survey will be continued for Broods B and C.

METHODS

Most species of June beetles fly and feed during the nights of the spring and summer. At this time one is able to pick them by hand or shake them from the branches of their host plant onto a large canvas. A flash light was used to hand pick them from the foliage in many cases. When collections were made during the daylight it was necessary to turn back dead leaves on the ground in woodlots and timber areas thus exposing the hiding beetles.

¹ Journal Paper J446 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 128.

Each collection from its host was tied in a cheesecloth bag, labelled as to locality, date of collection, and host. The bags containing the beetles were dropped into 70 per cent alcohol, where they remained until they were mounted for study.

COLLECTIONS AND SPECIES

During the summer of 1935, collections of June beetles were made in 69 of the 99 Iowa counties. Table 1 gives the number of individuals collected from the various plants. Since collections were made in many cases from plants that were most readily reached the choice of food plants as shown in the table cannot be considered significant.

In the case of each species collected the date on which it was first taken is recorded and in most instances the localities and host plants are given. The following list is according to the total number of each species collected by various investigators up to the present time and is not arranged to show relationship or position.

Phyllophaga hirticula (Knoch)

Dubuque, Leon, McGregor, New Sharon, Ottumwa, Clarinda, Kellerton, Mason City, Keokuk, Muscatine, Ames, Waukon, Fayette, Decorah, Cresco, Osage, Lake Mills, Algona, Fort Dodge, Boone, Centerville, Bloomfield, Washington, Mt. Pleasant, Tipton, Clinton, Cedar Rapids and Tama.

Taken from willow, elm, cottonwood, ash, shingle oak, hackberry, walnut, hickory, and quaking aspen.

A very abundant form. May 6 to July 23.

Phyllophaga implicita (Horn)

This species was abundant in every county where collections were made.

It was collected from elm, willow, hazel brush, cottonwood, ash, shingle oak, white oak, hawthorne, hackberry, dogwood, gooseberry, walnut, hickory, prickly ash, quaking aspen, ragweed, thistle, curled dock, and lambs quarter.

The most abundant species found in Iowa during 1935. It could be collected in numbers from almost any willow, cottonwood or elm in the state. Found as early as March 15 and as late as July 23.

Phyllophaga tristis (Fabricius)

Waukon, Ames, Ottumwa, Bloomfield, Cedar Rapids, McGregor, Davenport, Farmington, Wapello, and Mt. Pleasant.

Taken from elm, cottonwood, ash, and white oak.

Collection of this species first made on May 18 and last on July 2.

Phyllophaga rugosa (Melsheimer)

Ames, New Sharon, Keokuk, Muscatine, Sioux City, Sidney, Marshalltown, Clarinda, Leon, Centerville, Bloomfield, Mt. Pleasant, Ottumwa, McGregor, Dubuque, Clinton, and Maquoketa.

Collected from white oak, cottonwood, hackberry, shingle oak, box elder, walnut, hickory, elm, willow, linden, and quaking aspen.

Rusoga was very abundant in all the localities where it was collected. Many more specimens than were obtained could have been gathered had they been needed. First found on April 28 and last on July 22.

Phyllophaga futilis (LeConte)

Ames, New Sharon, Keokuk, Muscatine, Sioux City, Sidney, Marshalltown, Chariton, Mason City, LeMars, Cedar Rapids, Vinton, and McGregor.

Host plants from which it was taken were wild cherry, plum, linden, elm, and bur oak.

This species was distributed rather widely over the state during 1935, but was not collected in large numbers in any one place. The first specimen was found May 10 and the last on July 16.

Phyllophaga fusca (Froelich)

Ames, Des Moines, Indianola, Ottumwa, Oskaloosa, Davenport, Muscatine, Columbus Junction, Keokuk, Boone, Farmington, Algona, Mason City, Webster City, LeMars, Waukon, Decorah, Centerville, Corydon, Leon, Mt. Pleasant, Bloomfield, Dubuque, Clinton, Grundy Center, Marshalltown, Tama, Vinton, Cedar Rapids, Tipton, Newton, Albia, and Chariton.

Host plants included elm, willow, cottonwood, plum, linden, hawthorne, white oak, and quaking aspen.

Fusca appears to be a rather common species found in widely separated areas over the state. Found as early as April 8 and as late as July 22.

Phyllophaga hornii (Smith)

Kellerton, New Sharon, Oskaloosa, Blakesburg, Bloomfield, and Leon.

Hornii was collected from hickory in all the localities where it was found during 1935, with the exception of six specimens taken at Blakesburg on shingle oak.

This species was not found in numbers at any time. It was first recorded during 1935 on May 7 and the latest date of collection was July 10.

Phyllophaga inversa (Horn)

Ames, Des Moines, Kellerton, Columbus Junction, Centerville, Ottumwa, and New Sharon.

It was collected from three host plants, namely—hickory, willow, and walnut.

Only a few individuals could be obtained from any one host plant. First noted on May 16 and last on July 13.

Phyllophaga anxia (LeConte)

McGregor, Postville, Decorah, Bloomfield, Dubuque, Fayette, Mt. Pleasant, and Clinton.

Collections were made from willow, linden, and quaking aspen.

This species was never taken in numbers. It was first collected on June 3 and the last collection was made on July 23.

[illegible]

Gooseberry	Walnut	Hickory	Prickly ash	Red oak	Quaking aspen	Linden	Plum	Birch	Cherry	Bur oak	Box elder	Other	Total ♂'s	Total ♀'s	Total	Dates occurring
6	1	1	2	1	210							60	10303	11720	22023	3-15 7-23
	1				169							8				
	2	2			58								6026	6295	12321	5-6 7-22
		17			60											
	7	3			370	19					9		3421	3507	6928	4-28 7-22
					298	21					27					
					10	12	4						222	216	438	4-8 7-22
					4	7										
													211	196	407	5-18 7-2
													26	40	66	6-2 7-5
						7							29	19	48	4-29 8-1
						9							20	19	39	6-3 7-23
										27			27	11	38	5-20 7-3
										11						
	2	3											22	17	39	5-16 7-13
		9														
					3								19	18	37	5-3 7-11
													15	18	33	6-20 7-10
		4														
		9				21							21	8	29	6-27 7-15
						8										
		10											13	14	27	5-7 7-10
		11														
	5												7	13	20	5-13 7-17
	9															
						2	3		1				12	6	18	5-10 7-16
						1	2			3						
											3		4	10	14	5-28 7-6
								3					5	5	10	6-10
								2								
													3	5	8	7-8 7-15
													4	1	5	6-17 7-1
													1	3	4	6-7 7-19
													0	2	2	6-27
													2	0	2	6-28
													1	0	1	6-11
	1												1	0	1	6-19
											1		1	0	1	June

Sub
Total20416 22143
42559

Phyllophaga vehemens (Horn)

Missouri Valley, Onawa, Sioux City, New Sharon, Farmington, Keokuk, Vinton, Cedar Rapids, and Tama.

Collected only from walnut and ash.

It was first taken on May 13 and last on July 17.

Phyllophaga drakei (Kirby)

Ames, New Sharon, Oskaloosa, McGregor, Postville, Decorah, Farmington, Fayette, Tama, Mason City, Chariton, Albia, Council Bluffs, and Farmington.

Taken from willow, quaking aspen, white oak, and hazel brush.

Collected first on May 3 and last on July 11.

Phyllophaga crassissima (Blanchard)

Keokuk, Mt. Pleasant, Columbus Junction, Muscatine, Missouri Valley, Council Bluffs, Ottumwa, and Davenport.

Collected from ash, birch, and willow.

This species was rather scarce in most localities and usually only one or two individuals could be found on any one host. Earliest occurrence was on June 2 and latest on July 5.

Phyllophaga fraterna (Harris)

Clinton, Dubuque, and Mt. Pleasant.

Occurred on but one host plant—hazel brush.

Collected on June 17 and July 1.

Phyllophaga ilicis (Knoch)

Postville, McGregor, Sioux City, Davenport, Clinton, Centerville, Keokuk, Mt. Pleasant, and Wapello.

Taken from ash and linden.

Found first on April 29 and last on August 1.

Phyllophaga micans (Knoch)

Ottumwa and Mt. Pleasant.

Micans was collected from ash and shingle oak.

Collected first on June 20 and last on July 10.

Phyllophaga crenulata (Froelich)

McGregor, Postville, Centerville, and Cedar Rapids.

Taken from the foliage of bur oak in all cases.

The earliest date of collection was May 20 and the latest July 3.

Phyllophaga prunina (Le Conte)

Ames, Ottumwa, Keokuk, and Dubuque.

This species was collected from the leaves of hazel brush and white oak.

It was found on four occasions and in each case only one specimen could be found on a plant. The earliest record of its occurrence in 1935 was on June 7 and the latest was July 19.

Phyllophaga marginalis (Le Conte)

McGregor.

Taken from hazel brush.

The only date on which this species was collected was June 28.

Phyllophaga nitida (Le Conte)

Postville, McGregor, Fayette, Ames, and Hampton.

In every case it was collected from the foliage of linden.

The earliest collection was made on June 27 and the latest on July 15.

Phyllophaga congrua (Le Conte)

Woodbury County near Sioux City.

The only specimen that could be found was on black walnut.

Apparently a scarce species as only one specimen was collected and that on June 19.

Phyllophaga balia (Say)

McGregor, Ames, Postville, and Dubuque.

It was found on two species of plants, namely—willow and box elder.

This species was collected on two occasions, May 28 and July 6.

Phyllophaga bipartita (Horn)

Farmington and Postville.

The only plant on which this species was found was white oak.

Date of collection, June 10.

Phyllophaga corrossa (Le Conte)

McGregor and Ames.

Crataegus was the only plant from which it was collected.

Collected on July 8 and July 15.

Phyllophaga fervida (Fabricius)

Van Buren County, near Farmington.

The host from which it was taken was elm.

Only one specimen was found and that on June 11.

Phyllophaga forsteri (Burmeister)

McGregor was the only locality where this species was collected. It was taken from white oak foliage. Found June 27.

Phyllophaga hirtiventris (Horn)

Lakeside Laboratory, Dickinson County, Iowa.

One specimen from light trap taken by Prof. H. E. Jaques.²

² The writer wishes to express his appreciation to Prof. H. E. Jaques for furnishing this record.

Collected during June, 1935. This species has not hitherto been reported in Iowa.

DISCUSSION

As shown in table 1 a total of 42,559 beetles was collected during the spring and summer of 1935. Twenty-six species were taken although not all of them occurred in abundance. *P. implicita*, *P. hirticula* and *P. rugosa* were the most abundant species. During the early part of the season males were most numerous, at mid-season males and females were about equally abundant, and as the summer progressed females became the dominant of the two sexes. It is interesting to observe that out of 42,559 specimens there were 20,416 males and 22,143 females.

Elm, willow and cottonwood were in many localities almost the only available trees that could be used for sampling; for that reason a larger number of individuals was taken from these trees than from any other hosts.

More species of beetles were found in eastern and southern Iowa in any particular locality than in the western and northwestern portions of the state. No doubt, the fact that there are more species of host plants, and a larger area in permanent bluegrass pasture in the eastern and southern portions somewhat accounts for this. It is possible also that weather conditions, especially rainfall, play an important role in the distribution of the various species.

Table 2 records all the species belonging to Brood A so far as known. In column I is included the species and number of each collected by Jaques in the flight season of 1923, and published in a paper by him (1926); column II records those listed by the same writer in a paper published in 1927, and represents specimens collected by him during the flight season of Brood A in 1926. Column III records the June beetles listed by Travis (1934), and includes those specimens Jaques recorded in the two previously mentioned papers as well as many others added by Travis and other collectors. Column IV lists those taken by the writer during the flight season of 1935. Four species other than are shown in table 2 have been recorded as occurring in Iowa, but the year in which the adults were taken is not available. For that reason it is impossible to assign them to any one of the three broods. The four are *P. quercus*, *gracilis*, *ephilida*, and *spreti*.

FIELD TRIALS WITH STOMACH POISONS

Many requests are received each year by the Iowa Agricultural Experiment Station for a method of controlling imago Phyllophaga during the flight period. Trees and shrubs of various species are sometimes entirely denuded by the feeding activities of the nocturnal species. Certain field trials were made during the flight season of Brood A in 1935 in the hope of learning the relative merits of several poisons. Although there are several thousand references to June beetles in the literature of entomology only a few touch upon the control of the adults by means of insecticides. Davis (1916) mentions paris green, lead arsenate, and similar arsenicals as being effective against the beetles when sprayed on the foliage of the trees. Later, Vickery and Wilson (1919) showed that lead

TABLE 2. *Brood A records for Iowa*

Phyllophaga	1923 I	1926 II	1932 III	1935 IV	III & IV
<i>hirticula</i> (Knoch)	517	2,568	41,084	12,321	53,405
<i>implicata</i> (Horn)	31	239	9,980	22,023	32,003
<i>tristis</i> (Fab.)	1	9	10,647	407	11,054
<i>rugosa</i> (Mels.)	90	569	2,993	6,928	9,921
<i>futilis</i> (Lec.)	407	1,370	2,062	18	2,080
<i>fusca</i> (Froel.)	100	5,234	937	438	1,375
<i>hornii</i> (Smith)		15	560	29	589
<i>inversa</i> (Horn)		123	507	39	546
<i>anxia</i> (Lec.)		42	352	39	391
<i>vehemens</i> (Horn)		23	255	20	275
<i>drakei</i> (Kirby)	7	15	211	37	248
<i>crassissima</i> (Blanch.)	12	123	157	66	223
<i>fraterna</i> (Harris)	62	134	206	5	211
<i>ilicis</i> (Knoch)	8	12	140	48	188
<i>micans</i> (Knoch)	13	31	91	33	124
<i>crenulata</i> (Froel.)	6	6	53	39	92
<i>prunina</i> (Lec.)		2	52	4	56
<i>marginalis</i> (Lec.)			53	2	55
<i>nitida</i> (Lec.)			25	29	54
<i>congrua</i> (Lec.)			44	1	45
<i>balia</i> (Say)		3	23	14	37
<i>bipartita</i> (Horn)		4	14	10	24
<i>corrota</i> (Lec.)			8	8	16
<i>fervida</i> (Fab.)		14	14	1	15
<i>forsteri</i> (Burm.)		1	3	2	5
<i>villifrons</i> (Lec.)			4		4
<i>barda</i> (Horn)		2	2		2
<i>hirtiventris</i> (Horn)				1	1

arsenate, both as a spray and dust, was rather effective against certain wingless species which were destructive to cotton plants. In Wisconsin, Fluke (1933) has demonstrated that lead arsenate sprays (2 pounds to 50 gallons water) will prevent to a large extent the defoliation of oak trees. Other than these field trials a few references deal with poison trials under laboratory conditions. Travis and Decker (1933) made a study of the value of calcium arsenate as a dust for beetles caged in a screened insectary. Concurrent with the experiments to be reported in this paper, Andre and Pratt (1936) studied the relative value of certain stomach poisons in the laboratory when compared on an M.L.D. (median lethal dose) basis. The following field tests were conducted to ascertain whether the relative values of the compounds used in the laboratory would parallel those tested under field conditions.

Four dusts—namely, paris green, acid lead arsenate, calcium arsenate, and sodium fluosilicate—were used. Each poison was mixed with bentonite so that when the dust was applied it consisted of 40 per cent poison and 60 per cent bentonite by bulk. The trees selected for the trials were bushy willows about eight feet high. Dusts were applied to the trees about five o'clock in the afternoon. A hand duster was used to apply these dusts and they were applied at two different rates: (1) A light applica-

tion, where 2 pounds of poison were applied to each tree, and (2) a heavy application, where from $5\frac{1}{2}$ to 6 pounds of dust were applied to the foliage of the tree being dusted. Untreated control trees were chosen adjacent to each series of treated trees. All the trees were located on level ground in a row about a mile long and all were about the same size. The only species of beetle used in these trials was *P. implicita*. Two other species were often taken in small numbers—*hirticula* and *fusca*—but data on these are not included.

The beetles usually migrated to the willows at about 8:30 to 9:00 p. m. and soon commenced feeding. Each tree was watched to see if any particular treatment would cause the beetles to leave. It was soon evident that trees dusted with paris green, acid lead arsenate and calcium arsenate caused a portion of the beetles to leave before their normal feeding period was completed. Two hours after the incoming flight to the trees treated with paris green and lead arsenate, only approximately one-third of the beetles remained. Calcium arsenate treated willows lost about 10 per cent of their population in the same two-hour period, whereas those treated with sodium fluosilicate lost none.

To gain some insight as to the mortality obtained where the beetles left the treated trees before their normal feeding period was over the beetles were collected from the foliage as soon as they stopped feeding and prepared to fly from the trees. Although not as many could be collected in this manner as where they were hand picked after feeding for four hours, the results obtained with those collected in this manner when compared with the others offer an interesting comparison with the results from the longer feeding period.

In all other instances the beetles were allowed to feed for about four hours and were then picked from the foliage of the various trees. Each series was kept in separate boxes and marked as to the treatment they received. Then the beetles were taken to the screened insectary where they were confined in rearing cages which had a two-inch layer of moist soil in the bottom. The cages were supplied with fresh willow leaves each evening. At intervals of 24, 48, 72 and 96 hours the number of living beetles was recorded and the dead were discarded. Table 3 shows the percentage of dead beetles where the light dosages were applied and table 4 records the mortality obtained with the heavy dosages at the re-

TABLE 3. *The toxicity of four stomach poisons to June beetles, using light applications under field conditions*

Time in hours	Sodium fluosilicate		Acid lead arsenate		Paris green		Calcium arsenate		Checks	
	Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval	
	♂*	♀*	♂	♀	♂	♀	♂	♀	♂	♀
24	5.0	4.1	10.2	8.4	14.5	10.7	8.6	4.1	2.7	1.8
48	7.0	6.4	19.6	13.7	24.1	19.0	10.7	5.2	5.1	4.9
72	11.0	7.4	24.7	16.7	31.1	23.1	13.8	8.0	7.2	5.0
96	14.8	9.5	30.7	18.6	38.3	28.1	16.5	10.9	8.4	6.0

* Each column is based on a sample of 250 individuals. This applies also to table 4.

TABLE 4. *The toxicity of four stomach poisons to June beetles, using heavy applications under field conditions*

Time in hours	Sodium fluosilicate		Acid lead arsenate		Paris green		Calcium arsenate		Checks	
	Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
24	10.3	4.1	40.7	15.2	42.0	24.0	22.2	9.7	3.0	1.5
48	17.5	8.2	52.4	20.3	58.8	33.7	32.3	16.9	4.0	3.8
72	24.7	11.2	57.5	25.0	74.7	41.9	40.2	22.5	6.6	5.0
96	25.7	12.2	60.9	27.7	81.4	44.0	42.2	26.3	7.8	5.5

spective intervals of time. Table 5 shows the mortality in the case of paris green, acid lead arsenate and calcium arsenate where the beetles were picked from the foliage as they started to leave the treated trees after various lengths of periods of feeding. As is shown in this particular table irregular numbers of adults were used. In the case of table 3 and table 4, each test and check lot consists of a sample of 250 beetles.

PARIS GREEN.

Both heavy and light applications of this compound (tables 3 and 4) proved to be the most toxic stomach poison to both males and females under field conditions. With the heavy dosage 81.4 per cent of the males were dead after a 96-hour period whereas 44.0 per cent of the females died in this same interval of time. The light application resulted in a 38.3 per cent mortality in the males and a 28.1 per cent in the case of the females.

As is recorded in table 5, 103 males hand picked from the foliage just as they were preparing to leave the trees treated with the heavy application of paris green, showed a mortality of 98.0 per cent in the 96-hour period. A total of 124 females collected in the same manner died to the extent of 93.4 per cent in the same length of time. This increased mor-

TABLE 5. *The toxicity of three stomach poisons to June beetles, hand picked from foliage as they started to leave*

Time in hours	Paris green		Acid lead arsenate		Calcium arsenate		Checks	
	Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval	
	103 ♂'s	124 ♀'s	97 ♂'s	102 ♀'s	83 ♂'s	90 ♀'s	250 ♂'s	250 ♀'s
24	51.2	47.0	60.9	53.4	38.7	36.9	3.0	1.5
48	86.8	78.9	90.1	87.2	60.8	49.6	4.0	3.8
72	94.2	87.6	98.2	91.6	82.7	70.1	6.6	5.0
96	98.0	93.4	99.7	97.1	84.1	76.8	7.8	5.5

tality over the other beetles may have been partially due, at least, to the fact that the beetles received a toxic dose of poison, became sickened by it, and started to fly back to their hiding place in the soil.

It is interesting to note that paris green was also the most toxic to both males and females under laboratory conditions, and this poison was least readily eaten by them (Andre and Pratt, 1936).

ACID LEAD ARSENATE

Of the four stomach poisons compared acid lead arsenate ranked second in toxic value when employed as a dust under field conditions. With the heavy application 60.9 per cent of the males and 27.7 per cent of the females were dead after a 96-hour period.

Where the beetles were picked from the foliage (table 5) just before they left the treated trees, 97 males showed a mortality of 99.7 per cent, whereas of 102 females 97.1 per cent were dead in 96 hours.

A light application of acid lead arsenate produced a 30.7 per cent mortality in the females, and an 18.6 per cent kill in the males in a period of 96 hours. Under laboratory conditions Andre and Pratt (1936) found acid lead arsenate more toxic than sodium fluosilicate to both males and females when the M.L.D.'s are compared. The present field trials show that these three poisons rank the same in comparative value as under laboratory conditions.

CALCIUM ARSENATE

This dust ranked third in effectiveness to both males and females. Heavy applications produced a 42.2 per cent mortality in a 96-hour period in males and a 26.3 per cent mortality in females. Light applications killed 16.5 per cent of the males and 10.9 per cent of the females in the same period of time.

Out of a total of 83 males hand picked from the foliage which was heavily dusted, as they were preparing to leave, 84.1 per cent were dead in 96 hours. With the 90 females collected in this manner there was a mortality of 76.8 per cent in the same period of time.

In laboratory trials calcium arsenate did not prove to be very toxic and an M.L.D. value was not determined (Andre and Pratt, 1936). The laboratory sample used, however, was one that had been analyzed and kept in a loosely stoppered bottle since 1931, whereas all four poisons used in the field were freshly opened packages of commercial insecticides.

SODIUM FLUOSILICATE

Under field conditions this compound was least effective of the four tried. As is pointed out previously in this paper, it does not repel the beetles as much as do the other three compounds and for that reason is not included in table 5. Under laboratory conditions it was much less effective than were paris green and acid lead arsenate, a point which was also demonstrated in these field tests. A heavy application of this compound killed 25.7 per cent, whereas a light application killed 14.8 per cent of the males. In the case of the females, 12.2 per cent were killed by the heavy and 9.5 per cent by the light application.

CONTROLS

The beetles used as checks in all these tests were picked from trees not treated with any poison. By reference to table 3 it is evident that 8.4 per cent of the males and 6.0 per cent of the females died by the end of the 96-hour interval. The checks used in table 5 were the same as those used for table 4. Table 4 shows that 7.8 per cent of the males and 5.5 per cent of the females which were used as controls died in this experiment.

PERCENTAGE OF BEETLES NOT FEEDING

While studying the toxicity of paris green, acid lead arsenate, sodium fluoride and certain other insecticides under the conditions of the laboratory, it soon became evident that not all the beetles offered the various poisons would feed on them. As the writer felt that under field conditions this would be a rather important factor, a series of tests with the beetles confined individually in stender dishes were conducted to establish the percentage of adults normally refusing to eat these dusts. All these experiments, as well as the field trials, were conducted during the month of June. In the laboratory experiments the poison dusts were used alone and not mixed with bentonite.

Two per cent of the females and none of the males offered untreated leaves in the control cages refused to feed. Of those offered leaves treated with paris green 36.8 per cent of the females and 43.1 per cent of the males refused to eat. Where leaves treated with acid lead arsenate were offered the beetles, a total of 28.1 per cent of the females and 35.6 per cent of the males refused to eat. In the case of sodium fluoride, a compound not tried under field conditions, 41.2 per cent of the females and 51.2 per cent of the male beetles did not feed. Each of these feeding trials is based on 200 beetles.

It should be emphasized here that these above-mentioned figures were established when using *Phyllophaga implicita* during June and the poison dusts were used without bentonite. The writer is of the opinion that these percentages would vary—perhaps greatly under certain conditions as a number of complex interwoven factors are tied up here. Among them are the following: (1) Species of *Phyllophaga* being used as the test insect, (2) time of the season, (3) weather conditions at the time and immediately preceding the experiments, (4) hunger of the beetles, (5) sexual maturity of the beetles, and other external and internal conditions that one is unable to control in field experiments. One could therefore expect to get different results at different times of the year and by using different species of beetles.

SUMMARY

1. A survey was made during the summer of 1935 in an effort to determine the distribution of the various species of Brood A, June beetles in Iowa.
2. Sixty-nine counties were visited and a total of 42,559 specimens comprising 26 species of beetles were collected.
3. *Phyllophaga implicita* occurred more abundantly than any other form, while *P. hirticula* ranked second and *P. rugosa* third.
4. Field experiments with four poison dusts were conducted, namely: paris green, acid deal arsenate, calcium arsenate, and sodium fluosilicate.

Each was diluted with bentonite to form a bulk mixture of 60 per cent bentonite and 40 per cent poison dust. Under field conditions their toxicity value ranked in the descending order named.

5. A large number of beetles fed for various periods of time and left the plants when the foliage was dusted with the first three poisons mentioned. When these were collected by hand picking just before they left, a larger kill was obtained than was the case where the beetles fed on the poisoned foliage for a four-hour period. This was attributed to the fact that the beetles received a toxic dose of poison, became sick, and started to fly back to their hiding places in the soil.

6. Results obtained with paris green, acid lead arsenate and sodium fluosilicate under field conditions closely paralleled those obtained with the same compounds under laboratory conditions.

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A FLAVOR CONSTITUENT OF BLUE CHEESE (ROQUEFORT TYPE)¹

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A desirable flavor developed in a dairy product through fermentation is ordinarily due to a combination of chemical compounds. The compounds giving a product its characteristic flavor are often present in such small amounts that their separation and identification are difficult. The problem is especially complicated with cheeses since different lots of a given variety may show considerable variation in flavor when all of them are reasonably satisfactory.

The cheeses in which *Penicillium roqueforti* or a closely related species is a normal ripening agent have a peculiar peppery flavor that is rather characteristic of this general type of product. The origin of this flavor has been considered by various investigators. In 1914, Currie (1) reviewed the early ideas along this line. From his investigations he concluded that caproic, caprylic and capric acids and their readily hydrolyzable salts have a peppery taste and are responsible for the characteristic effect of roquefort cheese on "the tongue and palate."

When the flavor of good quality blue cheese is carefully considered, with the idea of recognizing the various components, the fatty acids can ordinarily be detected but they do not appear to explain the flavor completely. The results herein reported indicate that another flavor constituent is also of importance with this type of cheese.

EXPERIMENTAL

In connection with studies on the action of *Penicillium roqueforti* on various lower fatty acids, each acid was added to sterile milk and the milk then inoculated with mold spores. Commonly there was a rapid development of mold on the surface of the milk at room temperature and volatile acid determinations on the cultures, after acidifying with sulfuric acid, indicated that the fatty acids had largely disappeared. With larger amounts of fatty acids the mold growth was greatly delayed.

A flask to which was added 600 ml. of milk, 0.3 ml. n-caprylic acid, and mold spores was of special interest since after several days at room temperature it showed no mold growth at the surface but had an odor suggesting the peppery odor of blue cheese. Later, mold growth developed and the odor disappeared. This general result was regularly obtained in trials with n-caprylic acid while trials with n-butyric, n-caproic and n-capric acids, using various concentrations of the acids, did not yield

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² The authors are indebted to Dr. Henry Gilman and Mr. Miles R. McCorkle for much assistance in the studies reported. Mr. McCorkle prepared the various derivatives of the unknown and known ketones and largely established the identity of the two.

the odor. Different strains of *P. roqueforti* produced the odor with caprylic acid but not with the other three.

The peppery odor developed on adding caprylic acid (0.3 ml.) and mold spores to diluted milk (600 ml.) when there was as little as 1 part of milk to 63 parts of water, but was not detected with 1 part of milk to 127 parts of water or with water alone. The odor was especially conspicuous when 1 part of milk was used with 5 parts of water since it developed rapidly under these conditions and the odor of the medium was less noticeable than when undiluted milk was employed. The addition of caprylic acid and spores to a solution of 3 gm. of dipotassium hydrogen phosphate per 600 ml. of water also yielded the odor after an incubation period of about 2 weeks; with this medium mold growth did not develop at the surface and the odor increased in intensity over an extended period.

When medium in which the peppery odor had been developed was steam distilled, the odor soon disappeared from the material in the distillation flask. Extraction with ether removed the odor from the distillate. After drying the ether extract with anhydrous sodium sulfate, it was allowed to evaporate and a small amount of liquid obtained. The peppery odor was very conspicuous in this liquid. The relationship to the odor in the original flask was most evident when a drop of the liquid was shaken with many times its volume of water; in such material the odor persisted for weeks at room temperature. Larger volumes of the liquid were prepared by distilling 5000 ml. portions of medium in which the odor had been developed by adding caprylic acid in the usual proportion, together with mold spores, and combining the distillates. Preparations from milk commonly had the odor of caprylic acid along with the peppery odor while a preparation from the phosphate medium that had been incubated for several weeks did not.

Since the peppery odor suggested an ester, a number of esters of caprylic acid were prepared. These included ethyl, n-propyl, n-butyl, n-amyl, and n-octyl caprylates. None of them had an odor resembling the peppery odor. Attention was next directed to the methyl ketones since certain of these have a conspicuous odor and the material obtained by ether extraction gave a ketone reaction. Various methyl ketones were obtained and on the basis of odor it appeared that the compound of interest was methyl-n-amyl ketone. Identification was then established as follows:

Trial 1. The liquid having the peppery odor was obtained from 20,000 ml. of diluted milk culture by steam distillation, extraction with ether, drying, and evaporation of the ether. The liquid (1.5 ml.) was distilled; after removal of a small amount of ether the temperature rose to 148° and about 1 ml. (Fraction 1) distilled between 148° and 150°. The temperature then went up rapidly and between 200° and 220° the remainder of the liquid distilled; this material had the odor of caprylic acid and was largely soluble in sodium carbonate solution. The 2,4-dinitrophenylhydrazone of Fraction 1 melted at 91-92° after a single crystallization from alcohol. It did not depress the melting point (92-93°) of the 2,4-dinitrophenylhydrazone of a known sample of methyl-n-amyl ketone.

Trial 2. A larger sample of the liquid (9 ml.) was obtained from 20,000 ml. of potassium hydrogen phosphate culture with the usual procedure. This was distilled; after the removal of the residual ether the temperature rose rapidly to 144° and about 4 ml. of the liquid came over at 146-148°. The boiling point of the known methyl-n-amyl ketone was also 146-148° with

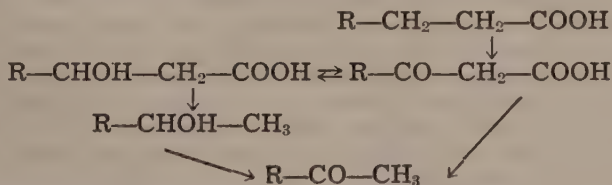
the apparatus used. The specific gravities of the unknown and known materials were 0.818 at 25°, the indexes of refraction were 1.4039 and 1.4036, respectively, at 25° and the semicarbazones melted at 121-122° alone or mixed.

Without any information as to the source of the liquids obtained from caprylic acid through the action of mold, various persons familiar with cheese described them as having the odor of blue cheese. This description was particularly convincing when given by someone especially interested in this type of cheese, either from the standpoint of consuming or marketing it.

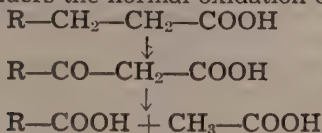
No odor suggestive of methyl-n-amyl ketone was obtained from heptylic acid through its addition to diluted milk containing spores of *P. roqueforti* but pelargonic acid yielded an odor suggesting this compound somewhat although it was still definitely different. In the original trials capric acid, which was added to the diluted milk in a liquid state, immediately solidified in relatively large masses (MP 31°). Additional trials were carried out in which the acid was added to the diluted milk, the milk warmed, and finally shaken during the cooling period to keep the acid finely divided. Under the action of the mold the capric acid yielded a compound with an odor somewhat suggestive of methyl-n-amyl ketone but still distinctly different. This odor was also slowly produced by adding the capric acid in the usual way and incubating at 37° rather than at room temperature.

The mold in the milk (diluted or undiluted) cultures yielding methyl-n-amyl ketone failed to develop in the normal manner. For a considerable period there would be no evidence of mold growth and when growth finally developed on the surface the odor of the ketone rapidly disappeared. In the phosphate medium masses of mycelium could be seen without any tendency to form a surface growth and one of the advantages of this medium was the persistence of the odor that developed.

The production of methyl-n-amyl ketone from caprylic acid suggests a beta oxidation together with the elimination of carbon dioxide from the carboxyl group. This type of change has been studied in connection with various products and presumably there are several possibilities from the standpoint of the exact steps involved. Stokoe (3) explains the formation of methyl ketones from fatty acids by molds as follows*:



He considers the normal oxidation of fatty acids to be



* The formation of the ketone from the keto acid would appear to be the more probable scheme because beta keto acids lose carbon dioxide so readily.

Stokoe believes methyl ketone is formed because the absorption of the poisonous fatty acids by the mold mycelium impedes respiration.

In a study of the methyl ketones in the oxidative decomposition of certain triglycerides and fatty acids from the standpoint of rancidity of coconut fat, Stärkle (2) considered the possibility of these compounds being important in dairy products, particularly in the rancidity of butter and in roquefort cheese. He concluded that the characteristic aroma materials in the ripening of cheese by molds are, in the case of roquefort cheese, methyl ketones instead of esters. Stärkle distilled roquefort cheese and obtained material (about 2 drops) that had an intensive odor of methyl-amyl and methyl-heptyl ketones. The mixed semicarbazone had a crude melting point of 105-107°. The amount was too small to permit separation and identification of the components. It was saponified with sulfuric acid and the odor of methyl-amyl and methyl-heptyl ketones noted.

GENERAL CONSIDERATIONS

The odor of methyl-n-amyl ketone can be detected in many lots of fine blue cheese and this compound appears to be an important flavor contributant. Along with this odor is the odor of various fatty acids, especially those of the volatile acids above butyric. In general, a conspicuous flavor of butyric acid is not as pleasing to most consumers as the flavor of the higher acids. Certain lots of cheese of satisfactory quality do not have an evident odor of the ketone although it may be present to some extent; in such cheese the desirable flavor appears to be supplied largely by the fatty acids.

The results obtained indicate that *P. roqueforti* can produce methyl-n-amyl ketone from caprylic acid. In blue cheese this acid evidently is freed from the fat by the lipase of milk or that produced by the mold. Since the mold can use the various lower fatty acids, it is probable that during the early stages of the ripening caprylic acid, together with the other volatile fatty acids, is destroyed as rapidly as formed. Later, conditions become less favorable for the normal action of the organisms, due to the lack of air, the diffusion of the salt to the interior of the cheese, the presence of the products of growth, etc., and the fatty acids accumulate. Some of the caprylic acid can be changed to methyl-n-amyl ketone and some of the capric acid may be changed to methyl-n-heptyl ketone; the latter transformation is suggested by the work of Stärkle (2). On the basis of the odor of blue cheese, methyl-n-amyl ketone would appear to be the important ketone involved. In the trials reported, the odor of methyl-n-amyl ketone disappeared from the cultures when active growth of the molds began so it is probable that a destruction of this compound also occurs in blue cheese. In cheese that does not have the odor of the ketone, the compound may have been destroyed or conditions may never have been satisfactory for its formation.

The butter defect which is commonly described as "roquefort flavor" very definitely suggests the odor of methyl-n-amyl ketone and undoubtedly this compound can be formed in butter through the action of molds under unfavorable growth conditions. Fat hydrolysis would yield caprylic acid and this could be changed to the ketone in quite the same way as in milk cultures or in blue cheese.

CONCLUSIONS

Methyl-n-amyl ketone is an important flavor contributant of blue cheese. The odor is conspicuous in many lots of cheese but is not evident in others although it may be present to some extent. This compound apparently is formed from caprylic acid through the action of *P. roqueforti* under unfavorable growth conditions.

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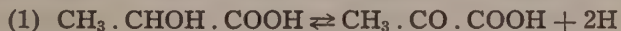
DISSIMILATION OF PYRUVIC ACID BY THE PROPIONIC ACID BACTERIA¹

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Virtanen (1923) was the first to study the fermentation of pyruvic acid by the propionic acid bacteria; from three molecules of the fermented acid he obtained one molecule of propionic acid and two each of acetic acid and CO₂. Van Niel (1928) concluded that acetaldehyde is not an intermediate, and suggested that the pyruvic acid is hydrated at the alpha-carbon and subsequently dehydrogenated to acetic acid and CO₂. Virtanen and Karström (1931) found that dried propionic bacteria were unable to ferment pyruvic acid except in the presence of phosphate. Support of the intermediate nature of pyruvic acid was given by Wood and Werkman (1934), who isolated it from the fermentation of glucose by sulfite fixation. They were unable to detect acetaldehyde. Later Erb, Wood and Werkman (1936) found pyruvic acid as a final product in the aerobic dissimilation of lactic acid by cell suspensions of propionic bacteria. They suggested that the bacteria catalyze the following reaction:



The present investigation of the dissimilation of pyruvic acid has been made as part of a general study of the dissimilation of proposed intermediates of the propionic acid fermentation. Only by making a critical study of intermediate dissimilation can a true picture of the whole process be obtained. Schemes of dissimilation founded wholly on the final products frequently lead to erroneous conclusions.

METHODS

The experimental procedure was that described by Erb, Wood and Werkman (1936). The Barcroft-Warburg respirometer (according to Dixon, 1930) and the macro-respirometer described by Wood, Erb and Werkman (1936) were used. The pyruvic acid (Eastman) was twice distilled under reduced pressure. A dilute solution neutralized to the proper pH with NaOH was sterilized by Seitz filtration. The acid was determined by iodoform titration before addition of buffer and bacteria. Succinic acid was determined, after distillation of the volatile acids, by extraction with ether and precipitation as the silver salt. Volatile acids, CO₂, lactic acid, unfermented pyruvic acid and oxygen utilized were determined as previously described by Erb et al. (1936). The bacterial suspension was not aerated previous to use.

Results given in tables 1, 2 and 3 were obtained by the Barcroft-Warburg technic with 2 ml. of medium containing 0.25 ml. of a suspension of 1 part of wet bacterial mass and 9 parts of water, 1.5 ml. of 0.15 M phosphate buffer and 0.25 ml. of 1.6 per cent neutralized pyruvic acid.

¹Supported in part by Industrial Science Research funds of Iowa State College.

TABLE 1. Carbon dioxide production and oxygen utilization in the aerobic dissimilation of pyruvic acid at different pH values*

Part I

Time in hours	Initial pH	Endogenous		Pyruvic acid		Net totals		Ratio CO ₂ : O ₂	Final pH
		O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂		
		mm ³	mm ³	mm ³	mm ³	mm ³	mm ³		
8	5.20	281.6	219.4	546.6	701.7	265.0	482.3	1.8	5.6
8	5.55	291.1	233.1	507.5	641.9	216.4	408.8	1.9	5.75
8	6.20	337.7	220.2	386.4	424.8	48.7	204.6	4.2	6.45
8	6.90	374.1	254.1	388.0	341.7	13.9	87.6	6.3	6.85

* By *P. arabinosum* (34 W) Age of cells = 72 hours + 37 hours in icebox.

Part II

8	5.85	183.2	178.1	300.7	440.4	117.5	262.3	2.2	5.50
8	6.30	179.0	156.3	268.9	374.0	89.9	217.7	2.4	6.00
8	6.70	176.4	155.6	263.8	313.6	87.4	158.0	1.8	6.35

* By *P. pentosaceum* (49 W) Age of cells = 72 hours + 8 hours in icebox.

In the macro-respirometer studies, five grams of wet bacterial mass were suspended in 500 ml. of medium consisting of 0.15 M. phosphate buffer at pH 5.6 and 0.7 per cent neutralized pyruvic acid (pH 5.6). Endogenous respiration values have been subtracted. Oxygen and CO₂ were the only endogenous values of quantitative significance. On a basis comparable to quantities given in table 4, the endogenous oxygen-uptake varied from 15 to 19 mM and the CO₂ from 19.3 to 23.2 mM. The anaerobic endogenous CO₂ was 7.9 mM. The time of incubation was 68 hours in experiments 1 and 2 and 32 hours in 3 and 4.

EXPERIMENTAL

The optimal pH range for the dissimilation of pyruvic acid was first determined (tables 1 and 2). The lowest pH used (5.2) in the aerobic

TABLE 2. Carbon dioxide production in the anaerobic dissimilation of pyruvic acid at different pH values*

Time in hours	Initial pH	Endogenous		Pyruvic acid		Net totals	Final pH
		CO ₂	mm ³	CO ₂	mm ³	CO ₂	
		mm ³		mm ³		mm ³	
8	5.25	64.6		174.4		109.8	5.15
8	5.65	45.8		571.8		526.0	5.50
8	6.00	57.7		402.4		344.7	5.60
8	6.25	42.5		238.6		196.1	6.10
8	6.80	31.9		166.9		135.0	6.50
8	7.50	41.6		75.3		33.7	7.00

* By *P. pentosaceum* (49 W) Age of cells = 72 hours + 8 hours in icebox.

experiments gave the largest oxygen-uptake as well as CO₂ production. Unpublished results show that the O₂ uptake and CO₂ evolved markedly decrease below pH 5.0. Table 2 shows the maximum CO₂ produced in the anaerobic dissimilation to be approximately pH 5.5. The same pH range was found optimal for lactic acid dissimilation (Erb et al. 1936). It is of interest that change in pH definitely affects the dissimilation, as shown by the variation in the CO₂:O₂ ratio, particularly with *Propionibacterium arabinosum* (table 1). Increase in pH resulted in an increase in the ratio. This effect is the opposite of that obtained with lactic acid in which the ratio decreased with increase in pH.

The oxygen uptake and the CO₂ production per hour (table 3) show a relative change as indicated by shifting of the ratio of CO₂:O₂. During the initial stage of dissimilation the ratio is high and then decreases. The conversion of pyruvic acid to acetic acid and CO₂ requires one atom of oxygen (equation 2) and the ratio CO₂:O₂ equals 2.

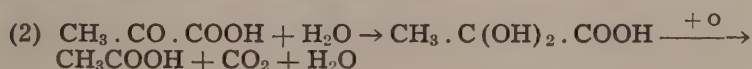


TABLE 3. Carbon dioxide production and oxygen utilization in the aerobic dissimilation of pyruvic acid

Initial pH 5.55; final pH 5.75

P. arabinosum (34 W)*

Time in hours	Endogenous		Pyruvic acid		Net rate per hr.		Net totals		Ratio CO ₂ :O ₂
	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	
	mm ³	mm ³	mm ³	mm ³	mm ³	mm ³	mm ³	mm ³	
1	74.1	46.3	99.6	123.2	25.5	76.9	25.5	76.9	3.0
2	46.8	32.9	54.5	100.4	7.7	67.5	33.2	144.4	4.3
3	54.5	38.6	78.2	89.7	23.6	51.1	56.8	195.5	3.4
4	36.4	34.3	70.7	82.9	34.3	48.6	91.1	244.1	2.6
5	24.0	25.3	62.6	70.1	38.6	44.8	129.7	288.9	2.2
6	20.1	19.1	56.2	65.8	36.1	46.7	165.8	335.6	2.0
7	16.2	18.4	46.3	61.2	30.1	42.8	195.9	378.4	1.9
8	18.8	18.2	39.4	48.5	20.6	30.3	216.5	408.7	1.8

Initial pH 5.85; final pH 5.50

P. pentosaceum (49 W)**

1	41.6	37.7	47.5	75.1	5.9	37.4	5.9	37.4	6.3
2	35.7	35.5	42.9	65.0	7.2	29.5	13.1	66.9	5.1
3	21.4	20.5	37.7	57.3	16.3	36.8	29.4	103.7	3.5
4	20.8	20.4	37.7	53.1	16.9	32.7	46.3	136.4	2.9
5	19.5	17.7	35.9	51.7	16.4	34.0	62.7	170.4	2.7
6	11.7	15.1	30.7	47.6	19.0	32.5	81.7	202.9	2.4
7	17.5	15.5	35.3	46.3	17.8	30.8	99.5	233.7	2.3
8	14.9	15.7	33.0	44.2	18.1	28.5	117.6	262.2	2.2

* Age of cells = 72 hours + 37 hours in icebox.

** Age of cells = 72 hours + 8 hours in icebox.

A ratio greater than 2 indicates that some additional substance other than O_2 is functioning as a hydrogen acceptor. A number of investigators have shown that the propionic acid bacteria do not form acetaldehyde and for this reason the decarboxylation of pyruvic acid to CO_2 and acetaldehyde is not considered. It seems probable that the high ratio is caused by part of the pyruvic acid acting as a hydrogen acceptor, in part replacing O_2 , to form lactic acid (reaction 1). Since this oxidation of pyruvic acid-hydrate to acetic acid and CO_2 involves no utilization of oxygen, a high ratio occurs. Inasmuch as propionic acid is the normal reduction product of anaerobic fermentation of pyruvic acid by propionic acid bacteria, it seems probable that the lactic acid is reduced in turn to propionic acid. On the other hand, the decrease in the ratio of $CO_2:O_2$ to approximately 2 indicates that the net results of the final conversion may have been that shown in equation 2, i. e., a quantitative oxidation of the pyruvic acid to acetic acid and CO_2 . If this is the case, it also involved oxidation of any propionic and lactic acids which may have been formed to acetic acid and CO_2 . The oxidation of lactic acid appeared possible but a similar oxidation of propionic acid did not seem probable since propionic acid usually has been considered a stable end-product of the fermentation. This indicated that propionic acid was not produced and that only lactic acid occurred which was formed in establishment of an equilibrium between pyruvic and lactic acids (Eq. 1). Furthermore, after equilibrium is reached, the oxygen acts as the hydrogen acceptor. This function of O_2 as an acceptor will bring about a decrease in the ratio of $CO_2:O_2$ and as an increasing amount of pyruvic acid is dissimilated the ratio will approach 2.

TABLE 4. Dissimilation of pyruvic acid by *Propionibacterium arabinosum* (34 W)

Num- ber	Pyruvic acid fer- mented per liter	Products per 100 mM of fermented pyruvic acid						O_2 util- ized per 100 mM of fer- mented pyruvic acid	Carbon re- covered	Redox index*
		Total vola- tile acid	Pro- pionic acid	Acetic acid	CO_2	Lactic acid	Suc- cinic acid			
	mM	mM	mM	mM	mM	mM	mM	mM	Pctg.	
1	82.4	90.9	17.2	73.7	98.5	1.0	0.0	46.1	100.1	0.94
2	81.1	92.5	11.1	81.4	95.4	0.6	0.0	38.6	94.5	1.01
3	83.4	95.0	26.3	68.7	66.7	0.0	1.6	4.0	96.5	1.00
4	73.5	97.8	36.3	61.5	58.0	0.7	0.1	anaero- bic	97.4	0.85

* Cf. Erb, Wood and Werkman. J. Bact. 13:595 (1936).

A perfect balance is indicated by an index $\frac{\text{Oxidized}}{\text{Reduced}} = 1.0$. The O_2 utilized is as-

sumed to be reduced to water and has, therefore, a reduction value of 2.0. Since the pyruvic acid is not neutral with respect to oxidation-reduction, as glucose is, but must be reduced by one molecule of hydrogen to be neutral, it is assigned a reduction value of 1 when used as a substrate.

In an endeavor to prove whether or not lactic acid occurs and to obtain more complete information concerning the mechanism of pyruvic acid dissimilation quantitative determination of the products was made with the macro-respirometer (table 4). The results show that there was no accumulation of lactic acid. Only final analyses are given in the table but experiments were run simultaneously and under similar conditions in which the lactic acid was determined at short intervals. In no case was there sufficient accumulation of lactic acid under either aerobic or anaerobic conditions to be of quantitative significance. This indicates that there may have been an oxidation of propionic acid; therefore the dissimilation of propionic acid was investigated. It was found that this acid as well as acetic acid is dissimilated in the presence of air with a utilization of O_2 and a production of CO_2 . These results will be reported in a separate communication. It is evident that aerobic dissimilation by propionic acid bacteria may result in a complete oxidation to CO_2 . The ratio of $CO_2:O_2$ is not determined alone by the initial conversion of the pyruvic acid but also by the subsequent dissimilation of the propionic and acetic acids.

The results in table 4 give a more complete picture of the changes. The determinations are reasonably accurate inasmuch as the carbon and oxidation-reduction balances are satisfactory with the exception of fermentation 4 in which the redox index suggests an error.

Fermentation 3 though conducted in the presence of air was predominantly an anaerobic dissimilation for little oxygen was utilized. In this experiment it was noted that the flask was not being shaken as vigorously as in fermentations 1 and 2 and the medium probably was not saturated with air. Fermentations 1 and 2 which utilized considerable oxygen, show an increased yield of acetic acid and CO_2 and a decrease of propionic acid.

The mechanism of aerobic and anaerobic dissimilation in the initial breakdown of the pyruvic acid are probably the same, involving pyruvic acid-hydrate (equation 2). Under anaerobic conditions part of the pyruvic acid functions as a hydrogen acceptor to be reduced to propionic acid while aerobically O_2 replaces the pyruvic acid as an acceptor, diminishing the propionic acid. Under weakly aerobic or anaerobic conditions (fermentations 3 and 4) equivalent quantities of acetic acid and CO_2 were formed but with strong aeration, the oxidation is more vigorous as shown by the production of CO_2 in greater quantities than the acetic acid. At present any explanation of the mechanism of this phase of the oxidation must be speculative. Evidence (Wood and Werkman, 1936^{1, 2}) indicates that propionic bacteria can synthesize succinic acid from acetic acid. Succinic acid is decomposed by resting cells with formation of CO_2 (unpublished data). The increased production of CO_2 under strongly aerobic conditions may result from a condensation of acetic acid and decarboxylation and oxidation of the condensation product.

SUMMARY

The dissimilation of pyruvic acid by cell suspensions of propionic acid bacteria has been shown to be most active at approximately pH 5.5. Propionic acid, acetic acid and CO_2 constitute the principal products. Acetic acid and CO_2 are probably formed by hydration and dehydrogenation of the pyruvic acid at the alpha-carbon, propionic acid probably by reduc-

tion of pyruvic acid through lactic acid. In aerobic dissimilation oxygen replaces the pyruvic acid as a hydrogen acceptor and there is a decreased yield of propionic acid. The mM of CO_2 were found equal to the mM of acetic acid under anaerobic or weakly aerobic conditions, but on strong aeration the mM of CO_2 were greater than the acetic acid. The increased CO_2 may result from oxidation of succinic acid which originates by condensation of two molecules of acetic acid.

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THE EFFECT OF PHOSPHATE FERTILIZERS ON THE REACTION OF GRUNDY SILT LOAM IN GREENHOUSE EXPERIMENTS¹

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The effect of fertilizers on the reaction of the soil has long attracted the attention of many investigators. Such materials as sodium nitrate and calcium cyanamide are known to leave basic residues, whereas, ammonium sulfate and potassium chloride have been found to leave acid residues in the soil. The continued use of any of these materials over a long period of years has been shown to influence considerably the reaction of the soil. The results of studies on the effect of phosphate fertilizers on the reaction of soils, however, have been quite variable. A neutralizing value for superphosphate has been reported (8) (13) (22). On the other hand some investigators (17) (24) (27) have found an increase in acidity following its use, while others (10) (15) (16) noted no change in reaction.

In some cases rock phosphate has appeared to have a neutralizing effect on acid soils (8) (27) (28) while in other tests no effect was noted. The latter conclusion was reached by a number of investigators (7) (19) (25) (26).

The claim has been made that rock phosphate can take the place of lime in acid soils. Rock phosphate may supply calcium as a plant nutrient and it may neutralize some of the soil acidity. Because of the difficulty of separating the nutritional effects of calcium-bearing materials from their neutralizing powers, investigators have been doubtful as to the relative importance of the two functions. Among those who believe that much of the benefit of calcium-bearing materials on plants growing in acid soils is due to the calcium available for plant nutrition are Robinson and Williams (20), Albrecht (1) (2), Whitson, Chapman and Hull (30) and some others (3) (5) recognize the importance of the nutritive value of calcium.

Pierre (19) found that the original reaction of the soil influenced the effect of different phosphate fertilizers on soil reaction as measured by pH over a period of 18 months. The more acid the soil, the greater was the neutralizing effect of the fertilizer. The different effects of the phosphates on soils varying in acidity were explained as due possibly to the different ways in which phosphate fixation occurs at different levels of acidity. He used superphosphate, rock phosphate, tricalcium phosphate, dicalcium phosphate, monocalcium phosphate and monosodium phosphate on three soils, one of high acidity, one medium, and one low. All the phosphates used reduced the acidity of the very acid soil. The neutralizing effects were less marked on the soil of medium acidity, monocalcium phosphate not affecting the reaction and superphosphate even increasing the acidity. On the slightly acid soil the neutralizing effects of tricalcium and

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dicalcium phosphate were very slight, whereas, monocalcium phosphate caused a slight increase in acidity and superphosphate an appreciable increase.

Cook and Connor (9) found considerable differences in the effect of rock phosphate and c.p. tricalcium phosphate upon the acidity developed by fertilizers when added to two soils, depending upon the method employed to determine the reaction. In one soil the two phosphates reduced the acidity measured by the Jones and Hopkins methods but when measured by percentage saturation and pH, the acidity was increased except in one case. In the other soil the tricalcium phosphate had no effect except when the Hopkins method was used and in this case the greatly reduced lime requirement was attributed to the fixation of aluminum by the phosphate.

EXPERIMENTAL

The primary aim of this experiment was to study the effect of rock phosphate on soil reaction and to attempt to determine whether or not rock phosphate could be substituted for lime by supplying the calcium required in plant nutrition. Rock phosphate was selected because of its use in a finely ground form as a fertilizer known as lime phosphate (14) and because of recommendations that rock phosphate be used in mixed fertilizers to correct some of the acidity present in them or resulting from their use (23).

An attempt was made to isolate any effects of the calcium supplied by the rock phosphate from its neutralizing effect. If some of the benefit from liming is due to the available calcium supplied, it is possible that rock phosphate may be substituted for lime by supplying calcium to the plant. In order to obtain some information on this point sodium phosphate was substituted for rock phosphate in several cases.

METHODS OF PROCEDURE

The pH measurements were made with the quinhydrone electrode according to the recommendations of Builman and Jensen (6). The Hardy and Lewis (11) lime requirement method was used. In the base exchange studies, measurements of exchangeable calcium and determinations of exchangeable hydrogen were made by the ammonium acetate method of Schollenberger and Dreibelbis (21).

In measuring base exchange capacity a modification of the procedure recommended by Schollenberger and Dreibelbis was followed. An open system so arranged as to keep the soil covered with the solution and to provide a continuous flow was developed and found to give comparable results with the closed system. Results obtained by this method compared closely with those obtained by Parker's (18) method.

Available phosphorus was determined by the 0.002 N sulfuric acid method of Truog (29). Harper's (12) modification of the phenoldisulfonic acid method was used to determine the nitrates. The official method (4) was followed in determining the calcium content of sweet clover, the magnesium nitrate method was used for total soil phosphorus and the Gunning-Hibbard method for total nitrogen.

The effects of different amounts of rock phosphate and of sodium phosphate in comparison with limestone on the reaction of Grundy silt

TABLE 2. *The effect of rock phosphate and sodium phosphate alone and with lime and of lime alone on Grundy silt loam in the greenhouse*

Treatment*	Mean pH **	Mean lime require- ment, lbs. per acre ***	Exch. H. M.E. per 100 gms. soil ****	Avail. PO ₄ p.p.m. *****	NO ₃ -N p.p.m. +	Exch. Ca M.E. per 100 gms. soil NH ₄ Ac. method ++
1. Check	5.02	6211	9.56	32.20	153.8	14.76
2. 500 lbs. rock phos.	5.06	6014	10.10	55.92	167.7	14.88
3. 1000 lbs. rock phos.	5.06	5989	11.38	82.50	168.0	14.78
4. 1500 lbs. rock phos.	5.06	6119	10.60	115.34	150.6	15.54
5. 2000 lbs. rock phos.	5.07	5782	10.59	142.36	156.3	16.04
6. 2500 lbs. rock phos.	5.08	5608	9.60	168.67	155.3	15.52
7. 3000 lbs. rock phos.	5.07	5616	11.55	194.03	161.9	16.03
8. 1000 lbs. rock phos. and 4 T. lime	6.63	1384	5.23	96.10	288.3	22.77
9. 2000 lbs. rock phos. and 4 T. lime	6.70	1463	5.81	147.16	277.3	23.11
10. Na ₂ PO ₄ = 1000 lbs. rock phosphate	5.28	5212	10.32	69.58	154.0	15.15
11. Na ₂ PO ₄ = 2000 lbs. rock phosphate	5.48	4955	8.68	102.37	181.8	15.74
12. Na ₂ PO ₄ = 1000 lbs. rock phos. and 4 T. lime	6.82	1238	2.67	89.20	299.6	23.77
13. Na ₂ PO ₄ = 2000 lbs. rock phos. and 4 T. lime	6.95	1172	2.05	132.37	310.8	23.22
14. 4 T. lime	6.72	1192	2.53	41.83	314.0	23.31
Highly significant difference (P = .01)	.02+	90				
Significant difference (P = .05)		71				

*—Each treatment in duplicate pots.

**—Average of 13 samplings over 20 month period.

***—Average of 9 samplings over 20 month period.

****—Average of 3 samplings over 20 month period.

*****—Average of 3 samplings over 12 month period.

+—One sampling after 16 month period.

++—Average of 3 samplings over 20 month period.

TABLE 3. *Analysis of variance of pH values of Grundy silt loam treated with different amounts of rock phosphate and sodium phosphate with and without lime and with lime alone*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	363	265.1973	.7306
Within date—treatment groups	182	.1907	.001
Between means of dates	12	26.99	2.25**
Between means of treatments	13	229.67	17.67**
Interaction	156	8.35	.05

** Means highly significant.

TABLE 4. Influence of different amounts of rock phosphate and sodium phosphate alone and in combination with lime and of lime alone on the lime requirement of Grundy silt loam in pounds per acre

Treatment-	Date of sampling									Treatment mean
	1-11	2-18	3-11	4-11	5-19	7-11	7-11	8-14	3-10	
	1933	1933	1933	1933	1933	1933	1933	1933	1934	
Check	6206	5280	6570	7884	6189	5931	5889	5995	5959	6211
500 lbs. rock phosphate	6111	5071	6074	6945	6214	6042	5830	5913	5924	6014
1000 lbs. rock phosphate	6111	4888	6104	6926	5796	6398	5749	5971	5960	5989
1500 lbs. rock phosphate	6165	4630	6078	7663	5821	6828	5789	5889	6216	6119
2000 lbs. rock phosphate	4142	4703	6115	6705	6214	6729	5667	5796	5971	5782
2500 lbs. rock phosphate	6098	4396	4470	5784	5772	6361	5633	5936	6030	5608
3000 lbs. rock phosphate	5989	4433	4630	5868	6018	6361	5655	5690	5901	5616
1000 lbs. rock phosphate + 4 T. lime	910	884	1350	1548	2002	1596	1519	1028	1624	1384
2000 lbs. rock phosphate + 4 T. lime	815	1253	1130	1597	1817	1818	1730	1531	1484	1463
Na ₂ PO ₄ = 1000 lbs. rock phos.	5018	4077	3684	4323	6079	6312	6006	5691	5726	5212
Na ₂ PO ₄ = 2000 lbs. rock phos.	4970	3905	4617	2997	6361	5465	5200	5399	5691	4955
Na ₂ PO ₄ = 1000 lbs. rock phos. + 4 T. lime	856	1130	1191	811	1400	1548	1473	1344	1391	1238
Na ₂ PO ₄ = 2000 lbs. phos. + 4 T. lime	978	1130	713	841	1424	1572	1496	1075	1321	1172
4 T. lime	964	848	762	958	1535	1609	1531	1192	1332	1192

Least mean difference highly significant = 90

TABLE 5. Analysis of variance of lime requirement of Grundy silt loam treated with different amounts of phosphate fertilizers with and without lime and treated with lime alone

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	251	1,258,911,091	5,015,584*
Within date-treatment groups	126	1,450,749	11,514*
Between means of dates	8	33,932,390	4,241,549*
Between means of treatments	13	1,163,997,898	89,538,300*
Interaction	104	59,530,654	572,410*

* Highly significant.

TABLE 6. Analysis of variance of lime requirement of Grundy silt loam treated with different amounts of rock phosphate (same data as used in table 4 but omitting the results for the soils treated with lime or with sodium phosphate)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between means of dates	8	34,140,566	4,267,571*
Between means of treatments	6	6,212,110	1,035,352*
Interaction	48	19,749,713	411,452*

* Highly significant.

TABLE 7. Exchangeable hydrogen content and total exchange capacity of Grundy silt loam by the ammonium-acetate method and the calculated amount of exchangeable bases and the degree of saturation

Treatment	7-11-33 (7 months)				11-23-33 (11 months)				8-11-34 (20 months)				Treatment means for exchangeable hydrogen M.E.
	Exch. H. M.E.	Exch. capacity M.E.	Exch. bases M.E.	Degree of saturation Pctg.	Exch. H. M.E.	Exch. capacity M.E.	Exch. bases M.E.	Degree of saturation Pctg.	Exch. capacity M.E.	Exch. bases M.E.	Degree of saturation Pctg.		
1. A Check	8.81	27.58	18.77	68.0	10.01	27.58	17.57	63.7	10.25	27.58	17.33	62.8	9.56
B	8.20	19.38	19.38	70.2	9.84		17.74	64.3	10.25		17.33	62.8	
2. A 500 lbs. rock	9.64	17.94	17.94	65.0	10.15		17.43	63.2	10.25		17.33	62.8	10.10
B phosphate	9.64	17.94	17.94	65.0	10.25		17.33	62.8	10.66		16.92	61.3	
3. A 1000 lbs. rock	10.87	16.71	16.71	60.5	10.25		17.33	62.8	12.92		14.66	53.2	11.38
B phosphate	10.25	17.33	17.33	62.8	10.25		17.33	62.8	13.74		13.84	50.2	
4. A 1500 lbs. rock	9.23	18.35	18.35	66.5	9.64		17.94	65.0	12.92		14.66	53.2	10.60
B phosphate	9.23	18.35	18.35	66.5	12.92		14.66	53.2	
5. A 2000 lbs. rock	9.23	18.35	18.35	66.5	10.25		17.33	62.8	13.74		13.84	50.2	10.59
B phosphate	8.61	18.97	18.97	68.8	8.20		19.38	70.3	13.53		14.05	50.9	
6. A 2500 lbs. rock	8.00	19.58	19.58	71.0	6.15		21.43	77.7	14.35		13.23	48.0	9.60
B phosphate	7.79	19.79	19.79	71.7	6.97		20.61	74.7	14.35		13.23	48.0	
7. A 3000 lbs. rock	7.79	19.79	19.79	71.7	11.89		15.69	56.9	14.76		12.82	46.4	11.55
B phosphate	7.38	20.20	20.20	73.2	12.71		14.87	53.9	14.76		12.82	46.4	
8. A 1000 lbs. rock	1.23	26.35	26.35	95.5	8.81		18.77	68.1	4.10		23.48	85.1	5.23
B phos. + 4 T. lime	1.03	26.55	26.55	96.3	9.84		17.74	64.3	6.36		21.22	76.9	
9. A 2000 lbs. rock	1.03	26.55	26.55	96.3	7.59		19.99	72.5	6.77		20.81	75.4	5.81
B phos. + 4 T. lime	2.46	25.12	25.12	91.1	10.25		17.33	62.8	
10. A Na ₂ PO ₄ = 1000	7.18	20.40	20.40	74.0	12.51		15.07	54.6	10.87		16.71	60.5	10.32
B lbs. rock phos.	6.77	20.81	20.81	75.4	12.71		14.87	53.9	11.89		15.69	56.9	
12. A Na ₂ PO ₄ = 2000 lbs.	5.95	21.63	21.63	78.4	11.89		15.69	56.9	8.68
B rock phos.	5.13	22.45	22.45	81.4	12.71		14.87	53.9	8.20		19.38	70.3	
12. A Na ₂ PO ₄ = 1000 lbs.	0	27.58	27.58	100.0	3.08		24.50	88.8	2.87		24.71	89.6	2.67
B rock + 4 T. lime	1.03	26.55	26.55	96.3	6.15		21.43	77.7	2.87		24.71	89.6	
13. A Na ₂ PO ₄ = 2000 lbs.	1.64	25.94	25.94	94.1	3.08		24.50	88.8	1.44		26.14	94.8	2.05
B rock + 4 T. lime	0.82	26.76	26.76	97.0	2.87		24.71	89.6	2.46		25.12	91.1	
14. A 4 T. lime	1.64	25.94	25.94	94.0	2.87		24.71	89.1	2.05		25.53	92.6	2.53
B	1.03	26.55	26.55	96.3	4.10		23.48	85.1	3.49		24.09	87.3	

Note: The values of base exchange capacity, exchangeable hydrogen and exchangeable bases are expressed in terms of milligrams per hundred grams of soil.

loam were studied in the experiment. Grundy silt loam, having a lime requirement of approximately $3\frac{1}{2}$ tons per acre and a pH of 5.30, was brought to the greenhouse, sieved through a $\frac{1}{4}$ -inch screen and 30 pounds of dry soil placed in each of fifty-six 4-gallon pots. The average phosphorus content of this original soil was 0.08 per cent and the average nitrogen content was 0.23 per cent.

The soils were treated according to the plan shown in table 1. The moisture content of the soils was adjusted to 50 per cent of the saturation capacity and maintained at that amount by additions of distilled water. Of the four pots per treatment, two were fallowed and used for sampling and two were seeded to sweet clover. A sub-irrigation system was used in watering the cropped pots. The fallow soils were sampled at intervals for determinations of pH, exchangeable hydrogen and base exchange capacity, exchangeable calcium, available phosphorus, nitrate nitrogen, and lime requirement.

RESULTS

pH DETERMINATIONS

The pH determinations were made over a 20-month period (table 1) and the treatment means are given in table 2. Wherever lime was used the pH was markedly increased. However, sodium phosphate with lime was more effective in raising the pH than was lime alone, whereas, rock phosphate with lime did not increase in every case where rock phosphate was used. The differences between the treatment means are of more consequence than if they were those of individual comparisons, yet it is doubtful if the differences are large enough to actually show a treatment effect. In order to test the significance of the variability resulting from these treatments an analysis of variance was made of the data. The total variability due to treatment was found to be highly significant (table 3).

LIME REQUIREMENT

Lime requirement data for the variously treated soils using samples taken at nine different dates are shown in table 4 and the treatment means are given in the second column of table 2. Lime and sodium phosphate each decreased the lime requirement of Grundy silt loam and there also seemed to be some tendency for rock phosphate to decrease the lime requirement of this soil. An analysis of variance (table 5) for all 14 treatments showed that there was a highly significant variability between treatments. This was also true of the variability resulting from the rock phosphate treatments alone (table 6). These results agree in general with those showing the pH. The higher amounts of rock phosphate generally produced lower lime requirements. Sodium phosphate was more effective than equivalent amounts of rock phosphate. The higher rate of sodium phosphate with lime was as effective as lime alone, actually more effective numerically, but only by a negligible difference. This was not true of the lower rate of sodium phosphate. Rock phosphate with lime was not as effective as lime alone.

Cook and Conner (9) attributed an increase in acidity following phosphate applications to nitrification. Since this might be the reason why rock phosphate with lime was not as efficient as lime alone, the nitrate

nitrogen content of the soil was determined. In table 2 it appears that no more nitrate was present where phosphates were used with lime than where lime was used alone.

BASE EXCHANGE PROPERTIES

Base exchange capacity, exchangeable hydrogen content, content of exchangeable bases and degree of saturation are given in table 7. An analysis of the data for base exchange capacity showed no significant differences which could be attributed to treatment. This would justify the use of the mean value for base exchange capacity which was 27.58 M.E. per 100 gms. of soil. Since the base exchange capacity did not vary with treatment, the base exchange content when figured as the difference between base exchange capacity and replaceable hydrogen, will vary inversely as does the hydrogen content. Hence only the results for exchangeable hydrogen are given in table 2. The marked effect of lime in lowering the content of exchangeable hydrogen is apparent. Sodium phosphate also decreased the exchangeable hydrogen content appreciably, but rock phosphate did not produce significant differences (table 8). As was the case when measured by pH and by the Hardy and Lewis lime requirement method, the higher rate of sodium phosphate used with lime was again found to be the most effective treatment.

Large increases in exchangeable calcium were found in all the limed soils by the ammonium acetate method (table 9) but an analysis of the data (table 10) revealed no significant differences between the means of the exchangeable calcium contents of the soils treated with rock phosphate alone.

GROWTH, YIELD AND CALCIUM CONTENT OF SWEET CLOVER AND AVAILABLE PHOSPHORUS AND NITRATE-NITROGEN CONTENT OF THE SOIL

In the early stages of growth there was a noticeably greater growth and greener color of sweet clover where rock phosphate was used than on the untreated soils. There was some tendency for the growth to be more vigorous as the rates of application increased. In contrast to all the other treatments a very poor stand and stunted growth of clover were obtained on soils treated with applications of sodium phosphate alone and with the lime alone. Sodium phosphate with lime and rock phosphate with lime produced only a fair growth which was not as good as that on some of the soils receiving higher rates of rock phosphate alone. Later

TABLE 8. *Analysis of variance of exchangeable hydrogen content of Grundy silt loam treated with different amounts of rock phosphate*

Source of variation	D.f.	Total sum of squares	Mean square
Total	41	208.82	
Within	21	3.92	0.187
Between means of dates	2	118.92	59.46**
Between means of treatment	6	22.45	3.74
Interaction	12	63.53	5.29**

** Mean square highly significant.

all the soils receiving some form of phosphate supported sweet clover crops of about equal vigor, whereas, the untreated soils produced poor crops, quite obviously below the average of the phosphate treated soils. However, differences in color were quite noticeable. The sweet clover in one of the untreated soils was light green, but the clover in the other one was normal. Clover in all the soils receiving different amounts of rock phosphate either alone or in combination with lime showed no chlorosis. The clover in the other treated soils including those receiving different amounts of sodium phosphate either alone or with lime and the soils receiving lime alone all showed distinct chlorosis. A few weeks later practically all the chlorosis had disappeared.

The first cutting of hay was made on June 11 and the second on August 5. The average dry weights of these cuttings are given in table 11. A statistical analysis of these data as presented in table 12 indicates that the average yields with the various treatments varied significantly.

The rock phosphate treated soils, except the 1500 pound per acre

TABLE 9. *Exchangeable calcium content of Grundy silt loam by the ammonium-acetate method of Schollenberger*

Treatment	M.E. Exch. Ca. per 100 gms. soil			
	Date			Treatment means
	7-11-33	11-33-33	8-11-34	
1. A Check	15.30	16.74	12.92	14.76
B	15.30	16.23	12.09	
2. A 500 lbs. rock phos.	17.57	15.09	12.71	14.88
B	15.71	15.51	12.71	
3. A 1000 lbs. rock phos.	16.54	15.30	12.82	14.78
B	16.12	15.09	12.82	
4. A 1500 lbs. rock phos.	16.95	15.71	14.68	15.54
B	15.71	15.50	14.68	
5. A 1000 lbs. rock phos.	16.33	17.36	15.09	16.04
B	15.71	16.74	14.99	
6. A 2500 lbs. rock phos.	15.30	16.95	14.78	15.52
B	15.30	15.71	15.09	
7. A 3000 lbs. rock phos.	15.30	15.71	14.99	16.03
B	15.71	16.12	13.33	
8. A 1000 lbs. rock phos. + 4 T. lime	23.15	22.12	23.36	22.77
B	22.74	21.91	23.36	
9. A 2000 lbs. rock phos. + 4 T. lime	24.39	22.94	23.15	23.11
B	23.56	21.70	22.94	
10. A $\text{Na}_2\text{PO}_4 = 1000$ lbs. rock phos.	14.88	15.63	14.68	15.15
B	15.09	15.63	14.99	
11. A $\text{Na}_2\text{PO}_4 = 2000$ lbs. rock phos.	14.88	16.74	14.88	15.74
B	15.71	17.16	15.09	
12. A $\text{Na}_2\text{PO}_4 = 1000$ lbs. rock phos.	23.25	25.01	22.84	23.77
B + 4 T. lime	23.77	23.15	24.49	
13. A $\text{Na}_2\text{PO}_4 = 2000$ lbs. rock phos.	23.36	23.36	23.15	23.22
B + 4 T. lime	23.36	23.15	22.94	
14. A 4 T. lime	23.36	23.98	22.84	23.31
B	23.15	23.05	23.46	

TABLE 10. *Analysis of variance in exchangeable calcium content of Grundy silt loam treated with different amounts of rock phosphate*

Source of variation	D.f.	Total sum of squares	Mean square
Total	41	71.91	
Within	21	5.95	0.283
Between means of dates	2	41.77	20.885*
Between means of treatment	6	8.22	1.37
Interaction	12	15.97	1.33

* Mean square highly significant.

treatment, produced highly significant increases in yield. Although some variability occurred in the results from the 500, 1000, 1500, and 2000 pound rates, rates of 2500 and 3000 pounds per acre produced mean yields greater than any of the other treatments by highly significant amounts. Likewise, the 2000 pound rate caused a significant increase in mean yield above all the lesser rates except the 1000 pounds per acre. All the treated soils yielded more sweet clover than the untreated soil. Hence,

TABLE 11. *The yield of sweet clover and the calcium content of sweet clover on Grundy silt loam*

Treatment	Yield in gms. ave. of 1st & 2nd cuttings	Pctg. Ca in Sw. Cl. ave. of 1st & 2nd cuttings
1. Check	18.55	2.212
2. 500 lbs. rock phosphate	22.47**	2.096
3. 1000 lbs. rock phosphate	19.17	2.180
4. 1500 lbs. rock phosphate	20.47**	2.170
5. 2000 lbs. rock phosphate	22.93**	2.088
6. 2500 lbs. rock phosphate	24.97**	2.291
7. 3000 lbs. rock phosphate	24.95**	2.026
8. 1000 lbs. rock phosphate + 4 T. lime	25.72*	2.392
9. 2000 lbs. rock phosphate + 4 T. lime	26.68*	2.362
10. Na_2PO_4 = 1000 lbs. rock phosphate	26.51	1.998
11. Na_2PO_4 = 2000 lbs. rock phosphate	26.84	2.014
12. Na_2PO_4 = 1000 lbs. rock phosphate + 4 T. lime	28.53**	2.282
13. Na_2PO_4 = 2000 lbs. rock phosphate + 4 T. lime	31.79**	2.328
14. 4 T. lime	28.87	2.438
Highly significant difference ($P = .01$)	3.83	
Significant difference ($P = .05$)	2.53	

* Significantly greater than the yield of any treatment above it in column.

** Highly significantly greater than the yield of any treatment above it.

it may be said that applications of rock phosphate increased the yield of sweet clover in most cases to a highly significant extent and that there was a tendency for this increase to be proportional to the amount of rock phosphate added.

The soils receiving lime or sodium phosphate produced mean yields of sweet clover significantly greater than that on the untreated soils. Rock phosphate with lime increased the yield over that from equivalent amounts of rock phosphate alone by significant amounts. One thousand and 2000 pounds of rock phosphate per acre with 4 tons of lime and the equivalent applications of sodium phosphate did not differ from each other significantly in respect to the mean yields of sweet clover.

The highest mean yield of sweet clover was produced by the higher rate of sodium phosphate in combination with lime. This yield was greater than that with either the lime alone or the lime with the lower rate of sodium phosphate by significant values and greater than the yields with any of the other treatments by highly significant amounts. All three of the last named treatments produced yields significantly greater than those resulting from any other treatment. It is interesting to note that the treatment producing the highest yield of sweet clover, namely, 4 tons of lime with sodium phosphate equivalent to 2000 pounds of rock phosphate, also produced the highest pH, the lowest lime requirement and the lowest content of exchangeable hydrogen.

DISCUSSION

Although each 500-pound application of rock phosphate did not result in a large increase in pH of the Grundy silt loam in greenhouse tests, there was a tendency for the higher amounts to be more effective (figure 1). This same tendency was even more noticeable when the reaction was measured by the Hardy and Lewis lime requirement method. Rock phosphate did not produce a significant difference in reaction of Grundy silt

TABLE 12. *Analysis of variance of sweet clover yields on Grundy silt loam*

Source of variation	Degrees of freedom	Sum of squares	Mean square
A			
Untreated soil and soils treated with rock phosphates only			
Total	13	424.92	
Within—treatment groups	7	99.26	14.18
Between means of treatments	6	325.65	54.88*
B			
Entire 14 treatments of Experiment			
Total	27	1813.0	
Within—treatment groups	14	298.8	21.34
Between means of treatments	13	1514.9	116.48**

* Significant

** Highly significant.

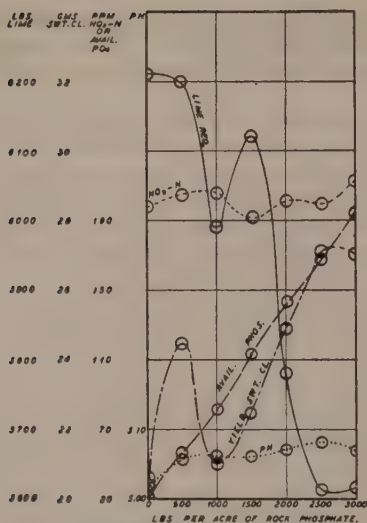


Fig. 1. Effect of rock phosphate on Grundy silt loam.

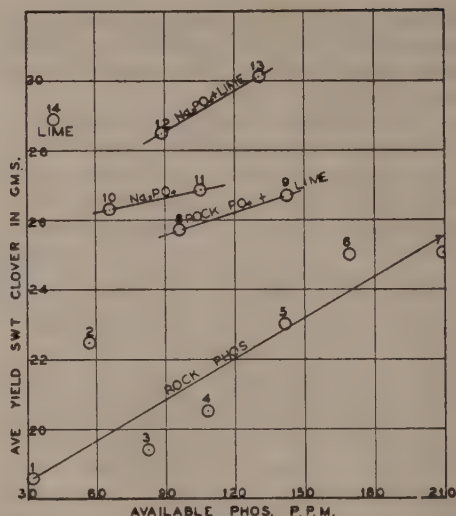


Fig. 2. The relation of available phosphorus to yield of sweet clover. Note: Numbers refer to treatment as in table 1.

loam, however, as measured by the base exchange capacity, the exchangeable hydrogen content, or the degree of saturation. Observations on the growth of clover revealed an early stimulating effect from all rates of application of rock phosphate, although there was not a great difference

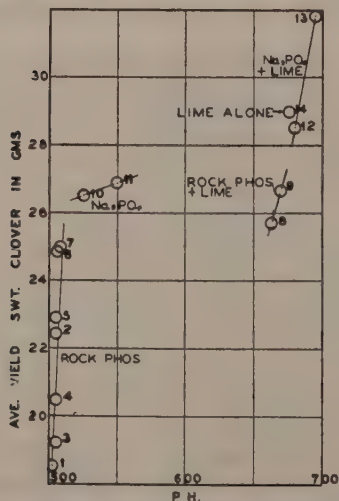


Fig. 3. The relation of pH to yield of sweet clover. Note: Numbers refer to treatment as in table 1.

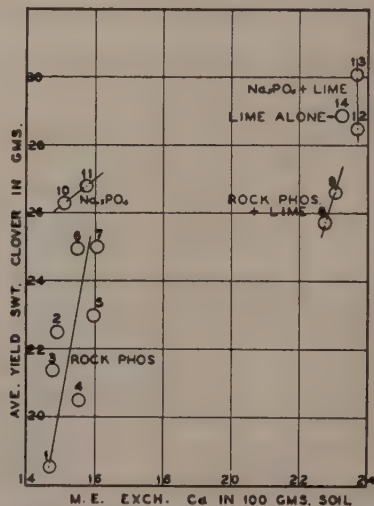


Fig. 4. The relation of exchangeable calcium to yield of sweet clover. Note: Numbers refer to treatment as in table 1.

between treatments. A significant increase in yield was obtained wherever rock phosphate was used, the two highest rates of application being associated with the highest yields. The percentage of calcium in the sweet clover plants was highest wherever lime was used. Rock phosphate produced no apparent effect on the calcium content of the sweet clover except in the case of the 2500-pound rate.

Except for 4 tons of CaCO_3 alone, the nutritional value of the phosphate added seemed to be an important variable affecting the growth of sweet clover on soils of similar pH and of similar exchangeable calcium content (figure 2). However, the kind of phosphate and the addition of lime with the phosphate affected the yield noticeably. The various treatments in which amounts of phosphate equivalent to that in 1000 pounds of rock phosphate were used did not show much variation in the amount of available phosphate in the soil following treatment but showed large differences in yields of sweet clover. This variability is greatly affected by type of treatment, that is, sodium phosphate with lime and lime alone produced the highest yields, sodium phosphate alone the next highest, rock phosphate with lime the next and rock phosphate alone the least of the treated soils. Lime applied with the phosphates in these tests did not depress the availability of the phosphorus.

Since the different combinations of fertilizers do not produce the same yields even when the amounts of available phosphate resulting from the treatments are approximately the same, it is apparent that other factors than the available phosphate supplied affected the yield of the sweet clover. That soil reaction is probably one of the most important of these factors may be seen in figure 3. However, the decrease in acidity produced by sodium phosphate does not seem to be large enough to account for all the effect of this material on sweet clover. The soils receiving lime and rock phosphate had much higher pH values than did the soils receiving only sodium phosphate but the yields of sweet clover were not noticeably different.

The effect of exchangeable calcium on yield is shown in figure 4. Why the very large increases in content of exchangeable calcium and in pH associated with the limed soils as compared with the soils receiving sodium phosphate alone did not cause greater increases in yield than they did is not known. The high figures for exchangeable calcium may be due to calcium dissolved from calcium carbonate by the reagents used. The rather small increases in yield with large increases in pH following liming may be due to the interaction between pH and other variables affecting yield. Large amounts of soluble phosphorus or calcium in the soil may augment the effect of pH on yield. In the case of the sodium phosphate treated soils the sodium may cause the increased yield of sweet clover previously pointed out.

A multiple correlation was made of the pH, the p.p.m. of available phosphorus, the p.p.m. of nitrate nitrogen, the exchangeable H and exchangeable calcium content of the soil, the percentage calcium in the crop and the yield of sweet clover in grams. The results (table 13) show that the amount of available phosphorus in the soil was not significantly correlated with yields of sweet clover. This does not prove, however, that the beneficial effect of rock phosphate on sweet clover was due to the lime supplied by the rock phosphate. This type of correlation is obviously due to the results produced by lime, a material which stimulated the growth of sweet clover greatly but added no phosphate.

A study of table 13 shows the high interrelation of the different factors to each other and emphasizes the difficulty of determining which of them was responsible for the effects of the different treatments. The very marked increase in both exchangeable calcium and pH and equally marked decrease in exchangeable hydrogen following applications of lime probably accounts for a large degree of the correlation between these variables.

When pH was isolated from the other variables it still had a correlation to yield that was highly significant. This was not true of the other factors studied.

The β values in table 13 show that pH was the most important independent variable affecting yield. Exchangeable calcium is next in importance, exchangeable hydrogen next and the effects of available phosphorus and nitrate nitrogen are negligible. In view of the very large effects produced by lime, these correlations doubtless show the effects of lime more than they do those of rock phosphate. Nevertheless, the same trends may be expected to hold true to a certain extent where rock phosphate is used alone.

SUMMARY AND CONCLUSIONS

Studies were made of the effect of finely-ground rock phosphate and sodium phosphate, each applied alone and in combination with lime and of lime alone on the reaction of Grundy silt loam in the greenhouse under uniform conditions. Changes in reaction were measured periodically by the quinhydrone electrode method, the Hardy and Lewis lime require-

TABLE 13. *The correlation of pH, exchangeable hydrogen, exchangeable calcium, nitrate nitrogen and available phosphorus content of Grundy silt loam and of the yield and calcium content of sweet clover*

	Nitrate-nitrogen	Exch. H.	Exch. Ca	Avail. P.	Yield Sw. Cl.	Pctg. Ca in Sw. Cl.
pH	.9880**	— .9610**	.9852**	— .0566	.8632**	.7454**
Nitrate N.		— .9654**	.9824**	— .1000	.8006**	.7816**
Exch. H			.9410**	.1674	— .8149**	— .7603**
Exch. Ca				.0107	.7736**	.7839**
Avail. P.					— .0774	— .1045
Yield Sw. Cl.						.3173

** Highly significant correlation.

.63 Least significant correlation factor.

.66 Least highly significant correlation factor.

β Values		
	Beta	r
β yield x Avail. P.	= +.0303	— .0774
β yield x % Ca in Sw. Cl.	= — .6892	+ .3173
β yield x Exch. H.	= +.5136	— .8149
β yield x Nitrate N.	= +.1684	+ .8006
β yield x Exch. Ca	= — .5667	+ .7736
β yield x pH	= +1.2550	+ .8362

ment method and by determining base exchange properties. In an attempt to isolate and evaluate the importance of the changes in reaction produced by the different treatments the variously treated soils were analyzed for content of exchangeable calcium, available phosphorus and nitrate nitrogen, and the effect of the different treatments on the yield and the calcium content of two crops of sweet clover was determined. The results of these studies may be summarized as follows:

1. Rock phosphate, sodium phosphate and lime did not cause significant changes in the base exchange capacity of Grundy silt loam.
2. Applications of 500 pounds per acre or more of finely ground rock phosphate produced slight decreases in the acidity of Grundy silt loam as measured by pH, and by lime requirement (Hardy and Lewis).
3. The small neutralizing effects of finely ground rock phosphate showed a tendency to increase with increased rates of application.
4. Finely ground rock phosphate caused highly significant increases in the yield of sweet clover on Grundy silt loam and there was a tendency for increased rates of application to produce higher yields.
5. There was a highly significant correlation between the reaction of Grundy silt loam and the yield of sweet clover. Likewise, the treatments producing higher pH values consistently produced higher yields regardless of the amount of available phosphorus present in the soil.
6. There was evidence that decreasing the acidity of Grundy silt loam as measured by pH, lime requirement and exchangeable hydrogen was more important in increasing the yield of sweet clover than were the changes produced in the content of replaceable calcium, nitrate nitrogen, or available phosphorus in the soil.
7. Sodium phosphate was more efficient in neutralizing acidity of Grundy silt loam than was rock phosphate applied in equivalent amounts on the basis of P_2O_5 content.
8. No evidence was found showing that rock phosphate could substitute for lime by supplying calcium to the plant. However, a high content of exchangeable calcium in the soil and a high percentage of calcium in the plant were associated with the highest yields of sweet clover. Yet where phosphate fertilizers were added there was no measurable increase in these constituents. Further studies on the effect of phosphate fertilizers on the soluble calcium content of the soil are desirable.
9. Four tons per acre of calcium carbonate did not noticeably depress the amount of soluble phosphorus in the soil.

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BIOLOGICAL ASSAY OF FEEDING STUFFS IN A BASAL RATION FOR COCCIDIUM-GROWTH-PROMOTING SUBSTANCE

I. PROCEDURE, YELLOW CORN MEAL, OATS, OAT HULLS, WHEAT, LINSEED MEAL, MEAT SCRAP¹

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The general methods employed in the biological assay of food materials for vitamin content are familiar to the biologist and biological chemist. After it had been demonstrated (Becker and Morehouse, 1937) that a number of food materials contain a thermostable (to autoclaving) principle which favors the development of coccidium *Eimeria nieschulzi* (Synonym: *E. miyairii*) in its rat host and that one food stuff (such as powdered yeast) may contain more of it than another (such as dry powdered pork liver), it seemed feasible to try to adapt the biological assay method to testing the ordinary feeding stuffs in a standard basal ration for their relative contents of this coccidium-growth stimulant, which elsewhere we have designated "coccidibios".

Owing to a number of difficulties, such as immunity considerations, lack of uniformity in vitality of cultures of the parasite, and variability in individual host susceptibility, it was obviously not feasible to attempt to determine units of coccidibios on the basis of the number of oöcysts of the parasite eliminated per gram of the tested material fed, in a manner comparable to the practice of estimating vitamin B or vitamin G units from the number of grams of body weight gained by a young animal per gram of a material fed as the exclusive source of the vitamin. A more promising alternative appeared to be determining a ratio between the number of oöcysts obtained when infected hosts were on one diet and the number obtained when hosts were on a standard diet, the requisite condition being, of course, that the test and reference series of experiments are carried on simultaneously and all other conditions of the experiment are as nearly alike for the two series as possible. The entire procedure finally adopted in order to derive an estimation of the relative coccidium-growth-promoting properties of feeding stuffs involves a number of important considerations, and will be explained in detail.

PLAN OF PROCEDURE

The Microörganism. The parasite employed throughout was *Eimeria nieschulzi* Dieben, which in former papers had been referred to as *Eimeria miyairii* (Cf. Roudabush, 1937). It parasitizes the gland cells of the small intestine of the wild brown rat and the tame variety. The particular strain was one obtained originally from a wild rat and carried on in white

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rats for several years, then inbred for four successive generations by infecting previously uninfected rats with single oöcysts. The oöcysts to be "cultured" were obtained from the caeca of heavily infected rats on the ninth day of the infection and washed three times by centrifugation. The last residue containing myriads of oöcysts was then mixed with 2 per cent potassium dichromate solution and poured into Petri dishes for the three-day sporulation period. The sporulated or infective stages were kept in an electric refrigerator. In order to maintain infective microorganisms with a high degree of vitality, the cultures were renewed about once a month.

The Host. The host employed throughout was the highly inbred Wistar A strain of albino rat. Every animal used had been raised in the laboratory from previously immunized females, and had remained uninfected from birth to the time of experimental infection. Ordinarily the average weight of the litter was between 60 gm. and 80 gm. when experimental feeding commenced. Previously mother and young had received a growing ration (Steenbock's) made up by pounds of the following materials: yellow corn meal, 76; linseed meal, 16; commercial casein, 5; ground alfalfa, 2; salt, 0.5; ground oyster shell, 0.5. Fresh milk was given every other day *ad libitum*. One or more litters were divided as evenly as possible according to sex and weight in order to obtain test and reference series for a particular trial.

The Diets. The reference or control diet in all cases consisted by parts of the following: beet sugar, 72; casein (unextracted), 15; salt mixture (Hawk and Oser), 4; lard, 3; cod liver oil, 2; Fleischmann's powdered yeast, 4. Rats grow moderately well on this diet throughout the duration of the experiment. A further reason for selecting 4 per cent as the amount of dry yeast was that this amount in the diet resulted in a moderate, but not excessive, growth of the parasite in the host after the infective dose of parasites had been administered.

The test or assay diets were made up by substituting the material to be assayed for the four parts of yeast and part of the beet sugar, so that the whole added up to 100 parts. The more concentrated feeding materials containing 30 per cent or more of protein were fed at the 10 per cent level, while less concentrated materials were fed at the 30 per cent level; for example, an oats diet would consist by parts of the following: beet sugar, 46; casein, 15; salt mixture, 4; lard, 3; cod liver oil, 2; ground whole oats, 30.

When the young rats had attained the proper weight they were placed on the test and control diets for 9 or 10 days previous to the date of the first experimental infection. During this time and subsequently they received only the special ration and water.

Infection. As previously stated, the first infective dose of oöcysts occurred at the end of the ninth or tenth day on the special diets, and another on the twelfth or thirteenth day. The doses of 2,000 organisms were administered directly into the stomach through a catheter while the rat was under ether.

Collection of Oöcysts. When the rats were infected as described, elimination of oöcysts commenced 7 days after the first infective dose, and practically ceased after 6 or 7 days. During this period of oöcyst elimination the rats were kept in individual cages floored with half-inch mesh hardware cloth and suspended over a flat pan holding 0.5 per cent "Kresol"

solution. The fecal pellets dropped directly from the rat into the disinfectant that prevented putrefaction.

Counting Oöcysts. The plan was to determine the number of oöcysts eliminated by test and reference rats during the entire infection, not the number per gram of feces. Two counts were made for each rat, one at the end of the third day of the collection period and the other after oöcyst elimination had practically ceased.

The pellets were disintegrated as thoroughly as possible in the collection pans by means of a masher made of a rubber stopper with a solid glass rod inserted into the smaller end for a handle. For further disintegration and mixing, the content of the pan was poured into a tin quart measure and thoroughly agitated with an electric mixer. Then it was poured into a glass cylinder and made up to 1,000 c.c. with water. After thorough mixing in another container, a sample of the mixture was quickly poured into a glass tumbler. The counts were made by means of a haemocytometer with a chamber of 0.9 cubic millimeter capacity over the ruled area. The actual number of oöcysts in two chambers, or 1.8 cubic millimeters, was determined by counting under the low power of the microscope. If the numbers of oöcysts in the two chambers did not check within about 10 per cent, two more counts were made. On the basis of these sample counts it was easy to calculate the approximate number of oöcysts passed by a discharging animal during the collection periods. The total number of oöcysts passed by a rat during an infection was the sum of the numbers passed during the two collection periods.

Treatment of Data. The data have been treated, admittedly, in a somewhat arbitrary manner. In each test the numbers of oöcysts passed by the rats on the 4 per cent yeast or reference diet have been taken as the standard for comparison with the other diets. On this basis it has been possible to define a yeast coefficient (F) as follows: F is the ratio of the number of oöcysts passed by 10 or more rats on a test diet to the number passed by 10 or more rats on the reference diet. Of course, the experimental procedure as previously outlined must have been closely followed for the coefficient to have any value.

It is also true that the coefficient expresses the relative coccidium stimulating properties of the rations as wholes, and not simply of the constituent variables; i. e., if the F value for a diet made up to 30 per cent with ground wheat were 1, this value could not be claimed to express simply a comparison of the coccidigenic properties of 30 parts of wheat and 4 parts of dry yeast, for even without wheat or yeast in the ration there would have been some development of the microorganism (V. Becker and Morehouse, 1935).

In the tables there appears a W -value representing the approximate mean ratio of the gains in weight of the assay series for the first 16 or 17 days on the special diets to the same for the reference series. The 16- or 17-day period will be referred to as the growth period of record.

ASSAY OF YELLOW CORN MEAL

The yellow corn meal used in these experiments was prepared by the Beaver Valley Milling Company, Des Moines, Iowa. It was fed at the 30 per cent level in the test ration. Corn is known to contain liberal amounts of vitamins A, B and E, to be rather deficient in vitamin G, and to be al-

most totally lacking in vitamin D. The latter was supplied in the cod liver oil. Since the casein was unextracted, there was some vitamin G from this source. The experiment was conducted in three trials.

The columns in table 1 headed "Wt. Gain" show the gains in weight made over a 16- or 17-day period, beginning the first day on the special diets and ending the seventh day of the infection. There is usually no weight loss due to coccidiosis before the eighth day when the infective doses are no larger than those employed in these experiments, no matter what the diet. Weight data for the remainder of the infection are not included in the table because there is often considerable irregularity attributable to the infection. The comments in this paragraph apply also to the tests of other feeding stuffs subsequently discussed.

The table shows that both series of rats made considerable growth gains, with but slight advantage in favor of the corn-feds, for the mean ratio of the gains of the test to the reference series (*W*-values) was 1.092.

The ratio of the number of cysts eliminated by the corn-fed series to the number eliminated by the yeast-fed series, or *F*-value, was .474. The logical deduction is that the basal ration made up with 30 parts of yellow corn meal has less than half the coccidium-growth-promoting properties of the same made up instead with 4 parts of Fleischmann's powdered yeast.

TABLE 1. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent yellow corn meal diets*

Rat Number	(1) Reference series		(2) Test series		Ratios (2) : (1)	
	Weight gain (gm.)	Oöcysts 10 ⁶	Weight gain (gm.)	Oöcysts 10 ⁶	Weight gains	Oöcysts counts
1	33	250	33	48		
2	25	168	44	56		
3	30	140	42	31		
4	33	127	48	147		
5	30	129	47	54		
Mean	30.2	162.8	42.8	67.2	1.417	.413
6	55	75	62	42		
7	53	64	43	73		
8	48	79	38	17		
9	43	63	33	7		
10	56	60	32	63		
Mean	51.0	68.2	41.6	40.4	0.816	.592
11	28	77	24	19		
12	36	142	29	64		
13	21	25	29	20		
14	28	62	40	12		
15	26	30	23	25		
Mean	27.8	67.2	29.0	28.0	1.043	.417
<i>W</i>					1.092	
<i>F</i>						.474

TABLE 2. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground hulled oats diets*

Trial	Rat Number	(1) Reference series		(2) Test series		Ratios (2): (1)	
		Weight gain (gm.)	Oöcysts 10 ⁶	Weight gain (gm.)	Oöcysts 10 ⁶	Weight gains	Oöcyst counts
1	1	37	407	34	89	0.99	.457
	2	60	310	44	285		
	3	died	died	66	118		
	Mean	48.5	358.5	48.0	164.0		
2	4	38	165	60	22	1.18	.381
	5	37	112	41	113		
	6	44	124	39	18		
	Mean	39.7	133.7	46.7	51.0		
3	7	34	144	26	249	1.39	.979
	8	30	243	35	176		
	9	26	184	64	134		
	Mean	30.0	190.3	41.7	180.3		
4	10	23	211	39	50	1.36	.564
	11	22	188	40	131		
	12	26	236	28	120		
	13	25	210	33	157		
	14	33	171	36	115		
	Mean	25.8	203.2	35.2	114.6		
W						1.25	
F							.591

It may be questioned whether in this case the difference between the effects of the two diets is actual or explainable on the basis of probability alone. In order to make a statistical test of this point, the data were subjected to an analysis of variance test following Snedecor's (1934) solution of a problem in which more than one item was involved in the classes. An "F"-value of 16.01 was obtained, whereas the tables of "F"-values show 7.81 as the value which would not be exceeded in random sampling from a homogeneous population once in a hundred trials. Therefore, the results of the two series do differ more than could ordinarily be expected if the diets were without effect.

ASSAY OF HULLED OATS

The hulled oats were obtained from a local feed store, carefully picked over to free them from foreign grains and oat hulls, and ground moderately fine in a coffee mill. The ground oats were fed at the 30 per cent level. According to Morrison's (1936) tables, oat kernels without hulls contain about 16.2 protein. Oats is known to be a good source of vitamins B and E, but not so good for vitamin G, and to lack vitamins A and D. The experiment was conducted in four trials involving 13 rats

in the controls and 14 in the test series. The weight and oöcyst enumeration data are shown in table 2.

Both series of rats increased steadily in weight on the diets during the growth period of record, but the oat-feds outgained the controls by about a fourth when the mean gains are compared.

Here, as in the case of the yellow corn meal tests, the grain recipients passed fewer oöcysts than the reference series, for the *F*-value was .591. The third trial was considerably out of line with the others, for there was little difference between the oöcyst counts for the two series, but it is to be expected on the basis of mere probability that such an outcome would occasionally be met with in trials involving only three rats in each series, even though the series represent different populations. The fourth trial shows definitely that the oat diet, which produced the greater weight gain, resulted in a lower oöcyst production.

ASSAY OF OAT HULLS

Finely ground oat hulls were prepared by the Quaker Oats Company upon the special request of the writers, and grateful acknowledgment for the courtesy is made at this place. They were fed at the 30 per cent level, making a rather bulky mixture. The rats became accustomed to the ration, however, and each actually made a mean gain of a gram and a half a day during the growth period of record. The data are presented in table 3. There was but one trial. The *W*-value of .688 shows that the test series grew better than two-thirds as fast as the reference series. The *F*-value of .494 indicates that the oat hulls did not exert a very pronounced stimulating effect upon the reproduction of the coccidium.

TABLE 3. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground oat hull diets*

Rat Number	(1) Reference series		(2) Test series		W	F
	Weight gain (gm.)	Oöcysts 10 ⁶	Weight gain (gm.)	Oöcysts 10 ⁶		
1	49	249	26	53		
2	42	247	36	153		
3	42	258	18	238		
4	26	237	28	132		
5	32	254	34	135		
6	36	299	16	74		
7	47	255	24	165		
8	39	296	28	114		
9	38	259	17	124		
10	31	198	36	78		
Mean	38.2	255.2	26.3	126.1		
					.688	.494

TABLE 4. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground whole wheat diets*

Trial	Rat Number	(1) Reference series		(2) Test series		Ratios (2): (1)	
		Weight gain (gm.)	Oöcysts 10 ⁶	Weight gain (gm.)	Oöcysts 10 ⁶	Weight gains	Oöcyst counts
1	1	25	206	36	178	1.535	1.065
	2	14	173	30	290		
	3	22	172	41	156		
	4	40	161	43	126		
	5	26	208	46	214		
	6	36	183	52	124		
	7	25	123	32	242		
	8	50	162		
	Mean	26.9	175.14	41.3	186.5		
2	9	16	80	35	75	1.721	0.779
	10	24	239	27	199		
	11	18	124	37	97		
	12	15	139	44	114		
	13	18	110	32	109		
	14	28	153	35	87		
	15	22	228	32	155		
	Mean	20.1	153.29	34.6	119.43		
W						1.628	
F							0.922

ASSAY OF WHOLE WHEAT

Whole wheat grains were purchased from a local dealer, picked over by hand to remove foreign grains and other materials, and finally ground in a coffee mill. The product was fed at the 30 per cent level. According to Morrison's (1936) tables, whole wheat contains about 13.5 per cent protein, considerable amounts of vitamins B and E, considerably less vitamin G, and lacks vitamins A and D. As in the case of the other test rations, the lacking vitamins were supplied in the cod liver oil. The experiment was conducted in two trials involving 14 rats in the reference series and 15 in the test series. The data are set forth in table 4.

The W-value of 1.628 shows that the wheat recipients made unusually good growth during the growth period of record, comparatively though not absolutely better than the series that was fed hulled oats.

The F-value of .922 indicates that the ration with wheat was approximately as stimulating to the reproduction of the coccidia as the ration with yeast. The two trials differ somewhat in respect to the ratios of the oöcyst counts for the two series; but both favor a rather high coccidiosis content in wheat.

It is to be recalled that Becker and Morehouse (1936) demonstrated the coccidium-growth-promoting property of wheat germ. We intend later to investigate wheat bran, wheat flour middlings and white wheat flour for the same property.

ASSAY OF LINSEED MEAL

The linseed oil meal purchased from a local dealer was said to be of the "old process" kind and to contain about 35 per cent protein. It was ground to a fine powder in a coffee mill, and fed at the 10 per cent level. According to Morrison (1936), linseed meal is an excellent protein supplement for most farm animals except poultry, but its vitamin content is not well known except for an appreciable amount of vitamin E. The data are represented in table 5.

A *W*-value of .80 tells the story of the weight gain on the rations for the growth period of record. It is somewhat lower than might have been expected for such a short period, but it is likely that while elements in the ration other than linseed meal supplied adequate amounts of vitamins A and D, vitamins B and G were exceedingly limited even though the casein was unextracted.

The *F*-value of .94 indicates that despite the poor rat growth on the linseed meal diet, there was practically as much coccidium-stimulant conferred on the ration by 10 parts of linseed meal as by 4 parts of powdered yeast.

TABLE 5. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 10 per cent linseed oil meal diets*

Trial	Rat Number	(1) Reference series		(2) Test series		Ratios (2): (1)	
		Weight gain (gm.)	<u>Oöcysts</u> 10 ⁶	Weight gain (gm.)	<u>Oöcysts</u> 10 ⁶	Weight gains	Oöcyst counts
1	1	23	122	25	142		
	2	34	147	29	91		
	3	30	135	32	114		
	4	45	69	29	95		
	5	42	130	20	228		
	Mean	34.8	120.6	27	134.0	0.78	1.11
2	6	45	98	36	90		
	7	53	75	47	84		
	8	57	90	41	36		
	9	51	154	33	60		
	10	42	83	45	119		
	Mean	49.6	100.0	40.4	77.8	0.81	0.77
<i>W</i>						0.80	
<i>F</i>							0.94

TABLE 6. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 10 per cent Swift's meat and bone meal diets*

Trial	Rat Number	(1) Reference series		(2) Test series		Ratios (2): (1)	
		Weight gain (gm.)	Oöcysts 10 ⁶	Weight gain (gm.)	Oöcysts 10 ⁶	Weight gains	Oöcyst counts
1	1	27	164	4	53		
	2	25	161	4	39		
	3	14	336	3	81		
	4	23	125	4	122		
	5	14	166	-5	78		
	Mean	20.6	190.4	2	74.6	0.1	.392
2	6	27	153	3	35		
	7	20	211	0	27		
	8	16	148	3	65		
	9	11	202	2	53		
	10	17	210	3	33		
	Mean	18.2	184.8	2.2	42.6	0.12	.231
W						0.11	
F							.311

ASSAY OF MEAT AND BONE MEAL

Swift's meat and bone meal (or meat scrap) was the product employed for the test. It was said to contain about 50 per cent protein, and for this reason was fed at the 10 per cent level. The writers have no information as to its vitamin content, but like tankage it probably contains some vitamin G but no vitamin B. The weight gains and oöcyst counts are recorded in table 6.

Weight gains were exceedingly poor on the meat scrap ration, for the table shows a W-value of only 0.111.

The F-value of .311 is likewise exceedingly low. It requires no formal statistical analysis to determine that the differences between the two series are significant. Evidently, then, meat and bone meal favors but slightly both rat-growth and coccidium-growth in the host.

DISCUSSION

It has been brought out clearly in a number of papers by Becker and Morehouse (reviewed in 1937) that the quantity of oöcysts eliminated during coccidian infection of the white rat is susceptible to modification through diet, and that one ration may have a greater stimulating effect upon the parasite's reproduction in the host than another, even though the rations differ in but one important respect. As an example of the latter statement, rats which consumed only a basal diet made up to 10 per cent with Fleischmann's powdered yeast eliminated more than 3 times as

many oöcysts as rats which received the basal ration with 10 parts of powdered pork liver and 4 parts of rice polish in place of yeast. The present study was undertaken in order to determine whether basal rations that differed in respect to supplements other than yeast and liver and rice polish might likewise exert differential influences upon coccidian infection. Then there were other questions for which more nearly complete answers were sought: Is the growth of the host the factor which regulates the intensity of the infection? Is vitamin G the growth stimulant? Is the bulk of the ration an important factor? The procedure (described above) for attacking these problems was based upon comparing oöcyst counts and weight gains for animals on the test rations with the same for animals on the ration made up to 4 per cent with Fleischmann's powdered yeast.

Yellow corn meal, hulled oats, oat hulls, and wheat were used at the 30 per cent level with strikingly different results. The respective F -values were .474, .591, .494, and .922. Thus, the yellow corn meal and oat hull rations exerted only half as much growth stimulating effect upon the coccidium as did wheat, and hulled oats something less than two-thirds as much. For the 10 per cent linseed meal and meat and bone meal rations the F -values of .94 and .31 indicated vastly different effects. Several other interesting comparisons are as follows: the 10 per cent linseed meal diet gave about the same F -value as the 30 per cent wheat diet; 10 per cent meat and bone meal gave a smaller F -value than 30 per cent oat hulls; and all the vegetable products gave larger F -values than meat and bone meal, the only animal product tested.

In a previous paper Becker and Morehouse (1937) have given reasons for their view that the growth of the parasite is not simply a function of that of the host; or, that common factors favor the growth of both host and parasite. The present work adds more evidence for that conclusion; for, on the one hand, we find the linseed meal ration with a W -value of only .80 and an F -value of .94, and, on the other, the wheat ration with a W -value of twice as much (1.628) and an F -value of about the same (.922) as the linseed meal ration. Also, conversely, a comparison may be made between the yellow corn meal ration with an F -value of .474 and the oat hull ration with an F -value of about the same, or .494; but the former has a W -value of 1.092 and the latter only .688, or slightly over three-fifths as much. These observations, in addition to those previously published, seem to prove definitely that the factor, or set of factors, that favors the parasite's growth is different from that which is necessary for good host growth. At least it is not possible to avoid such a conclusion at the present time.

Is the coccidium-stimulant identical with vitamin G? Becker and Morehouse (1937) have already stated reasons for considering it to be different. The present findings are not so conclusive as the former, but are in harmony with them. The case of linseed meal may be used as an example. According to certain authors, it is known to contain rather considerable amounts of vitamin B, i. e., B_1 . There is practically no information available regarding its vitamin G (or B_2) content. The limited growth we obtained with a basal ration made up to 15 per cent with unextracted commercial casein and to 10 per cent with linseed meal would indicate that vitamin G might have been the limiting factor. Nevertheless, the F -value for this diet was rather high (0.94).

One naturally ponders whether the bulk of the diet might not exert some mechanical effect upon the intestine that would affect the development of the coccidium. The experiments supply indirect evidence for the conclusion that the coccidium-growth effects observed are not to be accounted for on the basis of any such simple hypothesis. Referring again to Morrison's tables, corn has 2.2 per cent fiber; hulled oats, 1.9 per cent; whole oats, 10.6 per cent; wheat, 2.4 per cent; linseed meal, 8 per cent; and meat and bone scrap, 2.0 per cent. The amount of fiber in oat hulls was not shown, but it must be exceedingly high in view of the discrepancy between the percentages in hulled and whole oats. If the feeding stuffs are arranged according to fiber content, the following order is obtained: hulled oats, meat and bone meal, yellow corn meal, wheat, linseed meal, oat hulls. If now they are arranged according to *F*-values, they fall into the following order: meat and bone meal, yellow corn meal, oat hulls, hulled oats, wheat, linseed meal. In view of such a situation it is impossible to argue for or against either a hypothesis that fiber in the ration tends to provide conditions favorable for the development of the coccidium or one that fiber provides conditions that tend to restrict the parasite's activities.

All in all, these further studies tend to add evidence sustaining the hypothesis that there occurs in foods used by animals more or less of something of a chemical nature that stimulates coccidium-growth. This hypothetical principle was called *coccidibios* in a previous paper (1937). Further studies, however, will be necessary in order definitely to establish its existence and the nature of its action.

SUMMARY

A procedure for assaying feeding stuffs for their relative amounts of coccidium (*Eimeria nieschulzi*)-growth-promoting properties has been outlined. It is based upon a comparison of the numbers of the terminal stages of the protozoön discharged when the host (white rat) is consuming the tested material in a basal ration at the standard level (30 per cent for ordinary grains, etc., 10 per cent for concentrates) with the number discharged by the reference host on the basal ration made up to 4 per cent with Fleischmann's powdered yeast. It is a necessary condition of the experiment that the rats have not previously been infected and that the infective doses of the microörganism are the same in quantity and administered at the same time in test and reference series.

The ratios of the number of oöcysts from test animals to those from reference animals (*F*-values) for the rations made up with yellow corn meal, hulled oats, oat hulls, whole wheat, linseed meal, and meat and bone meal are, respectively, .474, .591, .494, .922, .94, and .311.

Attempts to correlate the *F*-values with weight gains made by the hosts during the first 16 or 17 days on the diets do not justify an assumption that the same qualities of the diet promote the growth of host and parasite. Neither is there a definite correlation between the bulkiness of the ration as measured by crude fiber and the development of the parasite as measured by oöcyst production.

The experiment further supports the earlier hypothesis that there occurs in feeding stuffs a coccidium-growth-promoting substance. In our earliest work it was considered to be vitamin G, but it is now considered

to be a distinct, though possibly related material. These conclusions apply to the rat infection.

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USE OF A DISCRIMINANT FUNCTION FOR DIFFERENTIATING SOILS WITH DIFFERENT AZOTOBACTER POPULATIONS¹

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After measuring several characteristics of each member of two or more groups, the investigator may wish to know if the groups differ significantly. The usual method is to test the significance of the difference between the group means, taking each character separately. But unfortunately, there is no way to combine the knowledge gained. The value of the information furnished by the several varieties may be different. Furthermore, correlation among the variates will make it inappropriate to treat the differences as independent.

Another method used is the Coefficient of Racial Likeness (6), which gives a single numerical measure of the whole system of differences. The Coefficient of Racial Likeness is made up of the sums of squares of the differences between the means of the variates in the two groups being compared, each squared difference divided by the corresponding variance. As pointed out by Karl Pearson, "The fundamental weakness of the Coefficient of Racial Likeness lies in the fact that it neglects the correlations between the characters dealt with."

Some recent articles (1, 2, 4, 7) present and illustrate a method for the differentiation of two or more groups which have been measured in several characters. This method has advantages over either of the methods just mentioned. The measured characters may or may not be correlated. They are combined to form a discriminant function which will give the maximum differences among the groups relative to the variance of the function within the groups. That is, a compound is chosen so that the "overlap" of the groups is a minimum. This method, as given by Professor Fisher, will be illustrated in the present article. A few modifications have been introduced in order to make available the methods of calculation described by Wallace and Snedecor (8).

A number of samples of Iowa soils were collected and examined for the presence of Azotobacter (5). One hundred of these samples were found to contain the organisms while 186 of them did not contain any. The pH, the available phosphate content and the total nitrogen content of the samples were determined. The data obtained required the calculation of the discriminant function in order to bring out the maximum difference between the two groups of soil samples. From the results obtained it will be possible (a) to determine whether these chemical soil measurements give significant information about the presence of Azotobacter and (b) to determine the relative value of these variates for such a discrimination.

Table 1 contains the data for the 100 samples which contained Azotobacter; table 2 the data for the 186 samples which did not. The pH of the individual samples ranges from 5.0 to 8.6, the amount of readily avail-

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able phosphates from 16 to 520 pounds per acre, and the total nitrogen content from 7 to 78 milligrams per 10 grams of soil. The mean value for each variate, for the samples in group I and group II, is given in table 3. The samples which contained *Azotobacter* have, on an average, a higher pH, more available phosphate and a larger total nitrogen content than the samples which did not contain the organisms. The mean differences, 1.408, 82.007, and 8.260, are given at the bottom of the table. The variates used:

$X_1 = \text{pH,}$

$X_2 = \text{the amount of readily available phosphate,}$

$X_3 = \text{the total nitrogen content,}$

are quite different in numerical size with correspondingly large differences in variance.

What weighted compound of the three variates will afford the maximum differentiation between the two groups of soil samples? Or, what coefficients of the linear function of the three variates,

TABLE 1. *pH*(X_1), *available phosphate content* (X_2) and *total nitrogen content* (X_3) of 100 samples of Iowa soils which contained *Azotobacter*

X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3
6.0	46	24	7.1	348	30	8.3	208	19	8.4	77	58
7.0	35	17	8.0	130	34	8.1	160	24	8.2	117	44
8.4	115	28	6.6	55	22	8.5	138	12	7.8	65	32
5.8	35	17	8.0	62	44	8.5	149	11	6.8	160	14
6.9	55	25	8.0	160	53	8.5	72	15	8.0	416	29
7.8	52	29	8.0	149	39	8.4	83	13	8.7	97	42
7.8	52	29	8.0	149	39	8.4	83	13	8.1	97	42
6.9	208	58	8.0	149	59	8.2	90	10	8.2	138	38
7.0	70	13	6.7	174	45	8.4	138	16	6.8	173	21
6.7	35	16	7.4	70	78	8.6	174	14	6.8	95	23
6.2	27	44	6.1	114	51	6.5	111	35	7.0	520	31
6.9	52	27	6.8	52	31	6.2	240	18	8.2	260	12
8.0	60	58	6.3	80	54	7.0	297	31	7.4	232	9
8.0	156	68	8.1	44	20	6.5	140	31	8.2	189	14
8.0	90	37	7.5	55	14	7.4	106	33	8.3	289	26
6.1	44	27	6.5	33	17	8.3	160	23	6.7	160	30
7.4	207	31	6.1	28	15	6.2	69	17	6.5	142	28
7.4	120	32	7.2	40	12	8.4	164	43	7.3	115	14
8.4	65	43	6.8	65	30	7.8	138	15	6.1	130	25
8.1	237	45	7.1	62	18	8.4	416	17	8.0	284	22
8.3	57	60	6.4	58	19	7.2	52	16	7.3	260	24
7.0	94	43	6.5	208	19	8.2	222	55	7.0	298	32
8.5	86	40	7.0	81	19	8.5	73	21	7.0	223	18
8.4	52	48	7.8	160	22	7.0	138	28	7.1	125	24
7.9	146	52	6.8	115	20	6.4	156	26	6.5	65	23

TABLE 2. pH (X_1), available phosphate content (X_2) and total nitrogen content (X_3) of 186 samples of Iowa soils which contained no Azotobacter

[illegible]

$$X = L_1 \frac{\bar{x}_1}{\sqrt{\sum x_1^2}} + L_2 \frac{\bar{x}_2}{\sqrt{\sum x_2^2}} + L_3 \frac{\bar{x}_3}{\sqrt{\sum x_3^2}},$$

will maximize the ratio of the difference between the means of the groups to the standard deviation within the groups? X is the weighted compound of the measurements of pH, available phosphate and total nitrogen. The quantities L_1 , L_2 and L_3 are the coefficients by which the respective measurements (each divided by the square root of the sum of squares *within groups*) of any individual soil samples should be multiplied in order to form its compound measurement X .

The difference between the means of X in the two groups is:

$$D = L_1 \frac{d_1}{\sqrt{\sum x_1^2}} + L_2 \frac{d_2}{\sqrt{\sum x_2^2}} + L_3 \frac{d_3}{\sqrt{\sum x_3^2}},$$

where d_1 , d_2 and d_3 are the mean differences between the three variates in the two groups. The problem is then to find the values of L_1 , L_2 and L_3 such that D is a maximum. The method involves the solution of a set of normal equations similar to those leading to multiple regression. Since there are two groups of observations, some of the calculations are like those which have become familiar in analysis of variance. The new features are to be described in some detail.

TABLE 3. *Number of samples, sums, means and mean differences for pH, available phosphate content and total nitrogen content*

Group		pH X_1	Phosphate X_2	Nitrogen X_3
I. With Azotobacter	Number Sum Mean	100 742.3 7.423	100 13312 133.120	100 2940 29.400
II. Without Azotobacter	Number Sum Mean	186 1118.7 6.015	186 9507 51.113	186 3932 21.140
Mean difference		1.408	82.007	8.260

In table 4 are recorded the computations leading to the pooled sums of squares and products within the two groups of soil measurements. In the line of totals, the entries are the sums of squares and products of the entire 286 observations in tables 1 and 2, no distinction being made as to group. In the lines for groups are put down the sums of squares and products of the group sums in table 3, calculated in the manner characteristic of analysis of variance. As examples, the entry for column X_1 in row X_1 of table 4 is,

$$\frac{(742.3)^2}{100} + \frac{(1,118.7)^2}{186} = 12,238.5321,$$

and for column X_2 , row X_1 ,

TABLE 4. Calculation of the correlation coefficients and the standard deviations within the groups

	pH X_1	Phosphate X_2	Nitrogen X_3
X_1	Total	158,287.7	45,671.1
	Groups	155,994.9808	45,472.6974
	Within groups	$\frac{\sum x_1 x_2}{\sum x_1^2} = \frac{2,292.7192}{10.5398}$ $\frac{\sqrt{\sum x_1^2} \sqrt{\sum x_2^2}}{S_{x_1}} = \frac{10.5398}{0.625422}$	$\frac{\sum x_1 x_3}{\sum x_1^2} = \frac{198.4026}{1.9078866}$ $\frac{\sqrt{\sum x_1^2} \sqrt{\sum x_3^2}}{S_{x_1}} = \frac{1.9078866}{.109743}$
X_2	Total	3,300,823.	597,415.
	Groups	2,258,023.8110	592,348.7355
	Within groups	$\frac{\sum x_2^2}{\sum x_2^2} = \frac{1,042,799.1890}{1,021.1754}$ $\frac{\sqrt{\sum x_2^2} \sqrt{\sum x_3^2}}{S_{x_2}} = \frac{1,021.1754}{60.595614}$	$\frac{\sum x_2 x_3}{\sum x_2^2} = \frac{5,066.2645}{175,161.7058}$ $\frac{\sqrt{\sum x_2^2} \sqrt{\sum x_3^2}}{S_{x_2}} = \frac{.028823}{.028823}$
	Total	198,980.	198,980.
	Groups	169,557.6344	169,557.6344
	Within groups	$\frac{\sum x_2^2}{\sum x_2^2} = \frac{29,422.3655}{171.5295}$ $\frac{\sqrt{\sum x_2^2} \sqrt{\sum x_3^2}}{S_{x_2}} = \frac{171.5295}{10.178404}$	$\frac{\sum x_2^2}{\sum x_2^2} = \frac{29,422.3655}{171.5295}$ $\frac{\sqrt{\sum x_2^2} \sqrt{\sum x_3^2}}{S_{x_2}} = \frac{171.5295}{10.178404}$

$$\frac{(742.3) (13,312)}{100} + \frac{(1,118.7) (9507)}{186} = 155,994.9808.$$

The differences in the third line are the sums of squares and products of deviations from means within the groups.

The calculation of the standard deviations and the correlation coefficients now proceeds in the usual manner (8, table 7a, page 32). As examples,

$$s_{x1} = \frac{\sqrt{111.0879}}{\sqrt{284}} = \frac{10.5398}{\sqrt{284}} = 0.625422,$$

$$r_{x1x2} = \frac{2,292.7192}{(10.5398) (1,021.1754)} = 0.213019.$$

The degrees of freedom used, 284, are those within the two groups, $(100 - 1) + (186 - 1)$.

It may be observed that the pooled standard deviations of these variates are very different, and that there is little correlation between the variates within the two groups. It is usually of interest to observe these statistics, and it takes little extra time to compute them. In addition, it has been found convenient to use the correlation coefficients in the solution of the normal equations which follow.

The correlation coefficients from table 4 are carried into table 5 where they are used to solve the linear function, X , which best discriminates the two groups of soils. The coefficients (L_1 , L_2 and L_3) required are proportional to the solutions of the equations,

$$\begin{aligned} r_{11}L_1 + r_{12}L_2 + r_{13}L_3 &= 1, \quad 0, \quad 0, \\ r_{12}L_1 + r_{22}L_2 + r_{23}L_3 &= 0, \quad 1, \quad 0, \\ r_{13}L_1 + r_{23}L_2 + r_{33}L_3 &= 0, \quad 0, \quad 1. \end{aligned}$$

Each expression, in turn, is set equal to 1 with the other expressions equal to 0.

Table 5 is worked in a manner similar to table 8 (page 36) in Wallace and Snedecor (8) except for the three back solutions. The k values obtained constitute the matrix in table 6. They will be used below to calculate the desired L coefficients.

Going back, now, to the mean differences given at the bottom of table 3,

$$\begin{aligned} d_1 &= 1.408, \\ d_2 &= 82.007, \\ d_3 &= 8.260, \end{aligned}$$

each difference is divided by the square root of its sum of squares within the groups, thus,

TABLE 5. Solution of equations, with each equation, in turn, set equal to 1 with the other equations equal to 0

	pH	Phosphate	Nitrogen			Sum
$k_{11} =$	1.00000 -1.00000	.213019 - .213019	.109743 - .109743	1.000000 -1.000000		2.322762 -2.322762
$k_{21} =$		1.000000 - .045377 954623 -1.000000	.028923 - .023377 .005546 - .005810	.0 - .213019 - .213019 + .223145	1.000000 .0 1.000000 -1.047534	2.241942 - .494792 1.747150 -1.830199
$k_{31} =$			1.000000 - .012044 - .000032 .987324 -1.000000	.0 - .109743 .001238 - .108505 + .109831	1.000000 .0 .0 - .005810 + .005881	2.138635 - .254907 - .010151 1.873608 -1.896510
$k_{12} =$ $k_{13} =$	1.059451	$k_{12} =$ - .222506 + .047398	- .109831 + .000638 + .012053	- .109831 - .223144 +1.000000		
$k_{22} =$ $k_{23} =$	- .222507	$k_{22} =$ 1.047568 - .223152	- .005881 + .000034 + .000645	- .005881 +1.047534 .0		
$k_{32} =$ $k_{33} =$	- .109831	$k_{32} =$ - .005881 + .001253	1.012224 - .005881 - .111084	1.012224 .0 .0		

$$\frac{d_1}{\sqrt{\sum x_1^2}} = \frac{1.408}{10.5398} = .133589,$$

$$\frac{d_2}{\sqrt{\sum x_2^2}} = \frac{82.007}{1021.1754} = .080306,$$

$$\frac{d_3}{\sqrt{\sum x_3^2}} = \frac{8.260}{171.5295} = .048155.$$

Multiplying the columns of the matrix in table 6 by these adjusted differences gives the values of the coefficients L_1 , L_2 and L_3 :

$$L_1 = (1.059451) (.133589) + (-.222506) (.080306) + (-.109831) (.048155) = .118374,$$

$$L_2 = (-.222507) (.133589) + (1.047568) (.080306) + (-.005881) (.048155) = .054118,$$

$$L_3 = (-.109831) (.133589) + (-.005881) (.080306) + (1.012224) (.048155) = .033599.$$

TABLE 6. *Matrix of multipliers reciprocal to the correlations within groups*

	pH	Phosphate	Nitrogen
pH	k_{11} 1.059451	k_{12} -0.222506	k_{13} -0.109831
Phosphate	k_{21} -0.222507	k_{22} 1.047568	k_{23} -0.005881
Nitrogen	k_{31} -0.109831	k_{32} -0.005881	k_{33} 1.012224

For the linear function, X , of the three variates, the difference between the means of X in the two groups is, as noted above,

$$D = L_1 \frac{d_1}{\sqrt{\sum x_1^2}} + L_2 \frac{d_2}{\sqrt{\sum x_2^2}} + L_3 \frac{d_3}{\sqrt{\sum x_3^2}},$$

$$\text{hence, } D = (.118374) (.133589) + (.054118) (.080306) + (.033599) (.048153) = .021777.$$

This value of D is the greatest difference that can be obtained from a linear compound of the measured characters of the two groups of soils.

In order to test the significance of the difference just computed, the sum of squares of the compound X is now analyzed into two parts, within the groups and between the groups. The *between groups* sum of squares is:

$$\frac{n_1 n_2}{n_1 + n_2} D^2 = \frac{(100)(186)}{100 + 186} (.021777)^2 = (65.035) (.00047424) = .030842.$$

This value is entered in table 7. The sum of squares *within groups* is the value of D, 0.021777. In determining the degrees of freedom between groups, note that in addition to the specific mean difference, two adjustable ratios have been used, making the three degrees of freedom. The analysis of variance is completed in table 7. The difference between the group means is highly significant, showing that the pH, available phosphate and total nitrogen content of the soils tested give significant information about the presence of *Azotobacter*.

TABLE 7. *Analysis of variance of the crude compound X between and within groups*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between groups	3	.030842	.01028
Within groups	282	.021777	.00007722
Total	285		

The relative value of these variates for discriminating between the groups is apparently indicated by the values of the coefficients,

$$L_1 = .118374,$$

$$L_2 = .054118,$$

$$L_3 = .033599.$$

It may be concluded from these results, therefore, that the pH, the content of available phosphate and of total nitrogen serve to significantly distinguish the samples of Iowa soils which contained *Azotobacter* from those which contained none of the bacteria. In addition, the results indicate that the presence of *Azotobacter* in Iowa soils may be most closely associated with the pH, closely associated with the available phosphate content of the soil and least associated with its total nitrogen content.

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THE ELECTRICAL CONDUCTIVITY OF COD LIVER OIL

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It has been known for many years that photographic plates when placed over cod liver oil are blackened, but there has been wide divergence of opinion as to the cause of this effect on the plate. It has been suggested that the plate is affected by some kind of radiation when the oil is oxidized, but it seems that no experiments have been performed which would either decisively prove or disprove this theory. Kugelmass and McQuarrie¹ have concluded that this phenomenon is merely another example of the "Russell effect." T. Avellar de Loururd² claimed to have shown that the blackening is caused by the formation of ozone when the oil is exposed to ultra violet light. Others have claimed that, in the process of oxidation, ultra violet light is excited which, in turn, affects the plate but later experiments have tended to disprove this theory. Some experiments by one of us have suggested the possibility of a radiation in the form of particles which are absorbed by a few centimeters of air. Botcharsky and Foehringer³ reported that photographic plates were affected by a radiation transmitted through the walls of glass tubes in which were sealed extracts of vitamin A and vitamin B, respectively. Experiments in this laboratory by Wissink⁴ have failed to check these results. Consequently, we decided to undertake an investigation to test whether there is any change in the electrical conductivity of the oil when irradiated by a strong source of ultra violet light. While making some preliminary determinations, it was noted that the conductivity of the oil in the apparatus varied rather irregularly before being exposed to the ultra violet. The effect was found also to be the same whether the apparatus was kept in the light or in total darkness. This behavior led us to extend our investigations to the variation of the resistivity of the oil under various conditions.

METHOD AND APPARATUS

The method of resistance measurement consisted in placing the oil in a conductivity cell, to be described later in the paper, in series with a galvanometer and a source of potential and determining the ratio of the current through the cell to the potential impressed across its terminals.

The arrangement of the apparatus for this part of the experiment is shown in figure 1 (a); G represents the galvanometer, which had a sensitivity of 2.1×10^{-10} amperes per mm. deflection at a scale distance

¹ Science, 62:87 (1925).

² Compt. Rend. Soc. Biol., 96:1321-1323 (1927).

³ Nature, 856 (1931).

⁴ Thesis, Iowa State College Library.

of 285 cms.; B is a 125-volt storage battery and P is a potentiometer arrangement by which any voltage from 0 to 124 volts may be impressed across the circuit; R is a resistance sufficiently high to protect the galvanometer against excessive current in case of a short circuit in the cell but negligible in comparison to the resistance of the oil; V is a good grade 150-volt D. C. voltmeter and C is the conductivity cell, various forms of which were used during the course of the experiment.

The source of ultra violet light used in the experiment on the photo-electric effect was an iron-nickel arc carrying a current of six amperes. This was enclosed in a metal case in one side of which a heavy brass tube containing a water cell was soldered. The windows in this cell were of quartz so that the passage of the ultra violet light from the arc to the specimen was unimpeded while the heat was absorbed by the water, thus insuring no change in the temperature of the oil because of the presence of the arc.

THE EXPERIMENT

As the experimental study extended to the investigation of several different problems, it may be discussed under five separate headings:

- A. The variation of resistance with temperature.
- B. The effect of exposure to air on the electrical resistance of the oil.
- C. Photo-electric effect.
- D. Dependence of resistivity upon vitamin A content.
- E. Miscellaneous.

A. THE VARIATION OF RESISTANCE WITH TEMPERATURE

The determination of the resistance of the oil was undertaken by measuring at different temperatures the current flowing through a thin layer of the oil confined between two brass electrodes across which a constant potential difference was impressed.

The original cell constructed for this purpose consisted of two brass electrodes in the form of combs of seven and eight teeth, respectively, so machined that, when fitted together as shown in figure 1 (b), the distances between surfaces was approximately .0064 cm. The area of cross section of the oil traversed by the current was approximately 6.75 cm². The cell was surrounded by a water jacket, the temperature of which could be altered as desired. The temperature of the oil was measured by a mercury thermometer graduated to 0.1° C., extending through the top of the jacket and resting on the brass electrodes below.

The results obtained with this apparatus were not sufficiently accurate to be of much value. As it was necessary to clean the apparatus after each trial and, at times, in doing so, to remove the spacers placed between the teeth to prevent short circuits, it was not possible to maintain a constant length of path between electrodes. Also, it was next to impossible to remove every particle of dust and lint from between the teeth when cleaning and these would often cause short circuits or at least decrease the length of the path of the current. However, this arrangement did serve well for preliminary work and in determining the order of magnitude of the resistivity which was found to be about 3×10^{10} ohms cms. at approximately 22° C.

For the succeeding work, the apparatus described in the foregoing paragraphs was replaced by one of simpler construction but capable of giving more accurate results. Two grooves, made accurately .02 inch apart, were machined into two blocks of hard rubber and these blocks were fastened into the ends of a hard rubber box. Two brass plates, .66 cm. by 3 cm., serving as electrodes were then fitted snugly into these grooves as shown in figure 1 (c). The brass container was replaced by an ordinary laboratory calorimeter fitted with a wooden cover and a larger water jacket was made up so that the temperature could be controlled more easily.

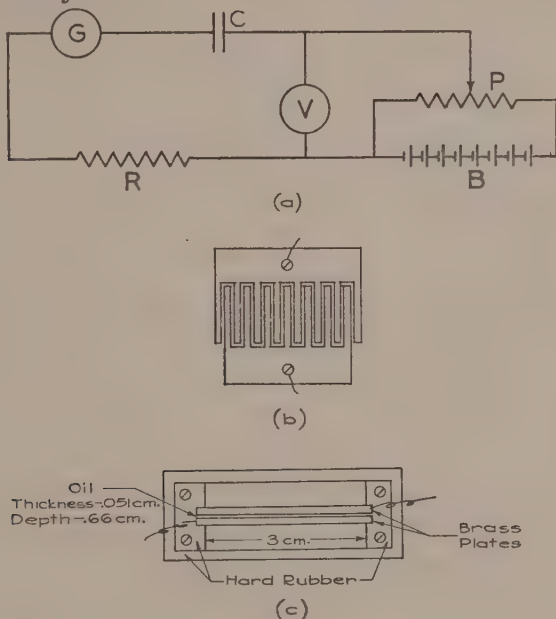


Fig. 1.

The general method of procedure was as follows: The oil was poured into the conductivity cell, the temperature raised to approximately 50°C . and the deflection of the galvanometer noted when a potential difference of about 120 volts was impressed on the cell. The temperature was then lowered a few degrees by adding ice to the water in the water jacket and the reading of the galvanometer was taken again. This was continued in small steps approaching 0°C . The temperature was then raised by approximately the same steps back to the original temperature, thus obtaining values of resistance for both falling and rising temperatures.

The graphs obtained when the resistance was plotted against the temperature closely resembled logarithmic curves. The shapes of the graphs obtained with falling temperature and rising temperature, respectively, were similar but the values of the resistances were usually different. A typical set of data is plotted in graphs I figure 2(a). Curve Ia represents the resistance changes during the first part of the experi-

ment as the temperature fell from about 43° C. to 7° C. The data for curve Ib were taken three or four hours after those for Ia as the temperature rose from 11° C. to 47° C., and those for Ic were, in turn, taken some time after those for Ib as the temperature fell from 47° C. to 8° C. These tests were made on Pure Test cod liver oil on March 25. Similar results were obtained in tests on Super D oil made on March 27 and March 28 as shown in graphs II and III, figure 2 (b), respectively.

It was at first thought that the differences of resistances shown by the earlier graphs of data from succeeding tests taken at short intervals, were probably caused by the lag in the readings of the mercury thermometer but, as the shifts in the curves in these cases seemed to be independent of the direction of the temperature change, it was decided that the errors in the thermometer readings could not account for the resistance differences noted. In order to further satisfy ourselves that this effect was not due to the lag in the thermometer, a copper-constantin thermocouple was substituted for the thermometer in the later experiments and the same results were obtained. The wide variations in the resistances noted in graphs I, II and III where the intervals between tests were comparatively long, proved quite conclusively that the cause must be looked for elsewhere. This effect will be discussed further in the next section.

Later experiments were performed in which the temperature of the oil was increased to approximately 90° C. The resistance-temperature curves shown in Xa and Xb, figure 3 (a), appeared to be approximately logarithmic over the whole range. To test this, the logarithms of the resistances were plotted against the temperatures. The graph then consisted of a series of several straight lines as shown in curves XIa and XIb, figure 3 (a). The dotted portions of these curves were obtained by a comparison with the data secured in several previous experiments over this range of temperatures. The breaks in these curves seemed, in nearly all cases, to be quite sharp, but the slopes of the different sections were not very consistent; that is, on one graph the slope of a given section was greater than that of the preceding section, while on another taken under as nearly as possible the same conditions, the reverse was true. This would seem to indicate a somewhat unstable condition at these higher temperatures. Also, these breaks did not appear at the same temperatures for different trials even though the specimens were taken from the same bottle. Judging from the behavior of the resistance itself as a function of time of exposure as discussed in the next section, the breaks could not necessarily be expected to occur consistently at any one temperature as the effect caused by the contact with the air was large at first and continued more slowly with further contact. In short, the time of change depended upon the previous treatment of the oil as well as upon the temperature.

The results of these experiments seemed to indicate that the change in temperature from 50° to 0° C. and return produced no permanent change in the resistance of the oil. In order to test this point further, a pair of long brass electrodes were inserted through a close fitting hard rubber stopper into a new bottle of oil. In this way, very much less air was admitted to the oil than was the case when the oil was poured into the open cell. When, in this experiment, the resistivity of the oil was

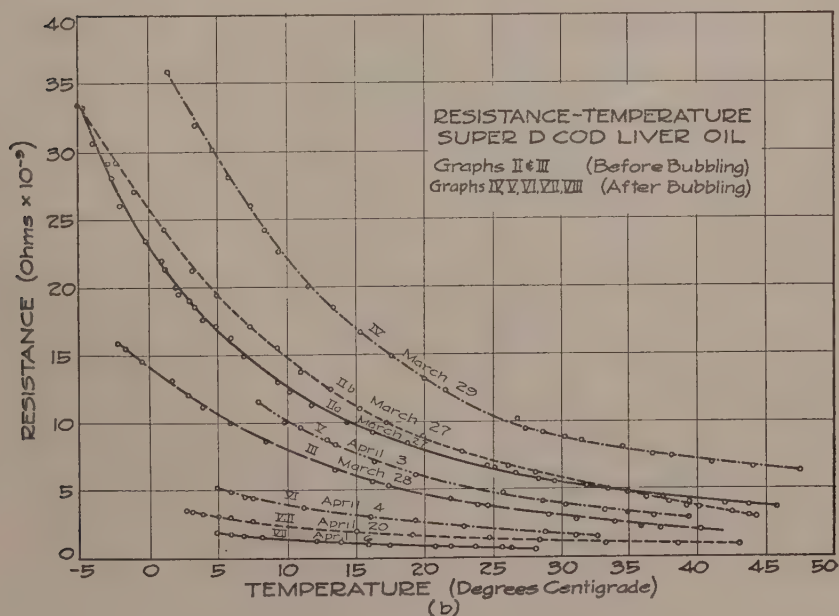
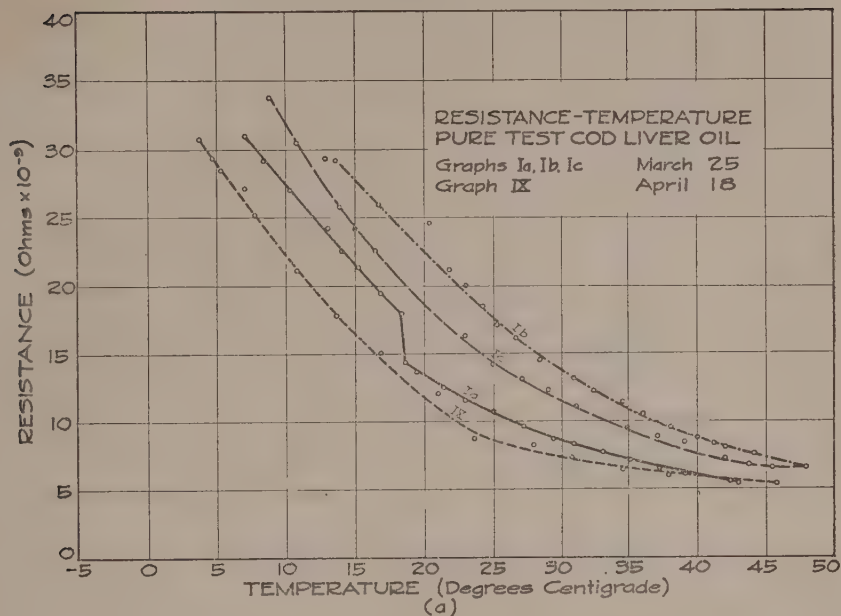
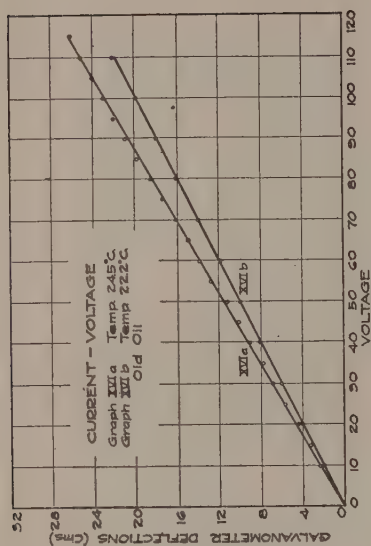
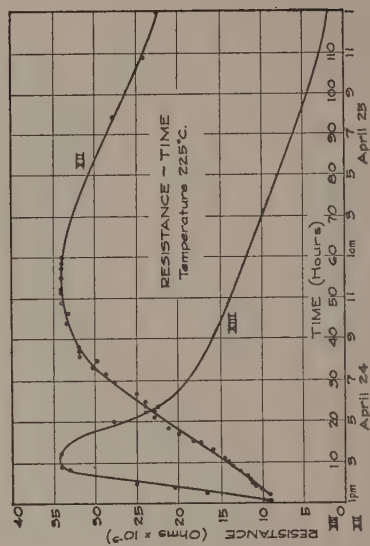


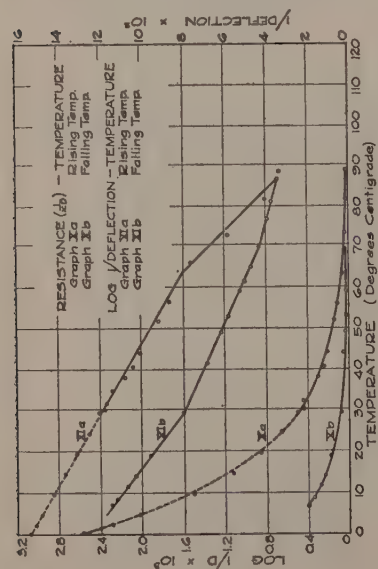
Fig. 2.



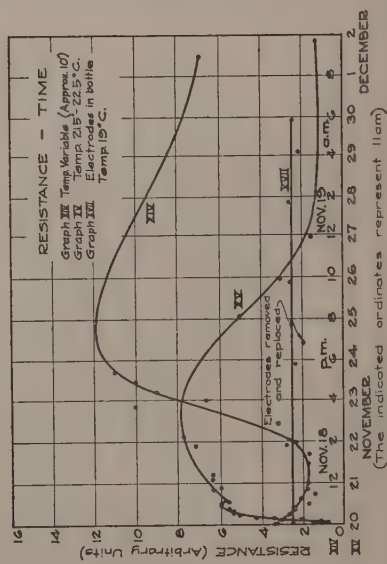
(b)



(d)



(a)



(c)

Fig. 3.

determined as the temperature was varied from 0°C . to 50°C ., or a few degrees above, no permanent change was found, which confirmed the above conclusion. When the temperature was raised as high as 90°C ., a permanent change in the resistivity was found, the resistance then being lower than before the oil was subjected to this high temperature. See graphs X and XI, figure 3 (a). It was inferred from this result that some chemical changes had taken place in the oil at these temperatures. If, however, after this oil had been allowed to cool and a sample then investigated in the usual manner, the shape of the resistance-temperature curve was the same as before but its position was shifted.

A test of a somewhat different nature was carried out on a sample of Super D cod liver oil. This was heated to 90°C . and air was bubbled through it for 35 minutes, the temperature being maintained at 88°C . to 90°C . When the resistance-temperature relation for a sample of this oil was determined, no change in the shape of the graph was found but this graph was found to lie considerably above the graphs obtained for the oil not subjected to this bubbling and high temperature, as is seen by a comparison of graphs II and IV, figure 2 (b). Thus again, while the actual resistance of these two samples was greatly different, the percentage change of resistance with change in temperature in each case was practically the same.

B. EFFECT OF EXPOSURE TO AIR ON THE ELECTRICAL RESISTANCE OF THE OIL

As noted above, it seems that some of the changes in the resistance of the oil were not due to temperature changes alone; because of this effect alone, the resistance of the oil at any temperature should be constant for all specimens taken from the same bottle and also it should not vary from day to day. A very striking example of this change is shown by the break in graph Ia, figure 2 (a), where work was interrupted for one hour but a practically constant temperature was maintained. The resulting resistance change amounted to about 25 per cent of the resistance at that temperature. Also, the rise in resistance as the oil was left in the apparatus exposed to the air as shown in Ib and the subsequent resistance drop with further exposure to air as shown in Ic is significant and led to further investigation as to the variation of the resistance of the oil as a function of the time of exposure to air, that is, possibly as a function of the oxidation of the oil.

These attempts to determine the variation of resistance with exposure to the air were made by allowing the oil to remain in the cell and noting its resistance at frequent intervals, while the temperature was kept constant. The results of a trial made on April 24-25, 1929, are shown in graph XII, figure 3 (d), from which it is seen that the resistance at first increased with time of exposure to air, reaching a maximum in 10 to 12 hours, and then decreased with further exposure. Observations made on April 29 on the same sample, which had been left in the apparatus continually, showed that in five days the resistance had dropped to a value less than that at the beginning of the test. These results are shown graphically in graphs XII and XIII, figure 3 (d), the first part of graph XIII being a reproduction of graph XII plotted to a larger time scale. This point was a little more carefully investigated later. A test made on November 18 showed that, for the first three hours

after the oil was taken from the bottle, there was a decrease in the resistance, then an increase for seven or eight hours, and then a decrease, as shown in graph XIV, figure 3(c). A series of determinations extending over a period from November 20 to December 4, are shown in graph XV, figure 3(c). It is to be noted that graph XIV represents less than the first day on graph XV. The variations were somewhat irregular during the latter states as shown in graph XV. These latter results seem inconsistent with those of April 24 near the beginning of the test, as no decrease was then noted. The oil on that date had, however, been in the apparatus for some time and it is probable that the first state noted in the later trials had been passed before the readings were started.

When the experiments mentioned in the foregoing with the electrodes inserted into the bottle of oil were being made, it was noted that whenever the electrodes were removed from the oil, cleaned and reinserted, the resistance for a short time was a little lower than before the removal of the electrodes. This could be accounted for in view of the results obtained on November 18 and 20 by the fact that a little air was carried between the electrodes into the oil, producing a small amount of oxidation. The data obtained with the electrodes immersed in a full stoppered bottle kept at constant temperature over a period of several days are shown in graph XVII, figure 3(c). It is seen that the resistance is quite constant under these conditions. As neither the length nor the cross section of the path of the current could be accurately determined with these electrodes, no attempt was made to calculate the resistivity from the data obtained with them.

That some of the changes noted in the oil did not require large quantities of air present was indicated by the fact that samples taken from the same bottle did not give the same results. For example, the results shown in figure 2(a) were for samples taken from the same bottle but on different dates, graphs I on March 25 and graph IX on April 18. The bottle had been kept stoppered between these dates but was probably only about three-fourths full so that about one-fourth pint of air remained in the bottle above the oil. A new bottle of oil was obtained and its contents divided among twelve smaller bottles which were tightly stoppered, wrapped in black paper to exclude any effects of light on the oil, and stored in a room at ordinary temperature. In this way, only a little of the oil was exposed to the air at any time. However, results obtained from samples from different bottles gave widely differing results. These results were probably due to the oxygen obtained in the pouring from one bottle to another and from bottle to apparatus, and, also, while standing in the apparatus a short time while preparations were completed for a test. It was shown in section A that when an excess of air was introduced into the oil by bubbling at high temperature, the resistance rose to about four times its former value. However, after this aerated oil had been allowed to stand for several days, its resistance dropped back to a value slightly below its former value, as shown in the graphs in figure 2(b).

The results obtained in different trials were so variable that an interpretation is very difficult. It seems from a general survey of the results, that a slight aeration of the oil produces a small decrease in the resistance, while a further aeration causes a very considerable increase. It is possible that the decrease in the resistance with sufficient aeration

is caused by the presence of acids produced by oxidation. Also, the breaks in the logarithmic curves described above may be due to the change in the composition of the oil when these acids are formed. The permanent lowering of the resistance produced in the oil when heated to high temperatures may also be due to the same cause.

C. PHOTO-ELECTRIC EFFECT

Two separate methods of investigating the photo-electric effect were employed. In the first, the original set of electrodes described under "apparatus" was employed, figure 1(b). Just enough oil was poured into the cell to fill the spaces between the teeth. As thin a layer of oil as possible was desired as the oil is nearly opaque to ultra violet light. When a constant temperature of the oil had been obtained, a reading was taken on the galvanometer in the circuit shown in figure 1(a), the oil irradiated, and the galvanometer deflection again noted. In the second method, a simple photo-electric cell was employed, consisting of a thin layer of oil spread on an aluminum plate which served as one electrode. The other electrode was a fine copper gauze placed about two millimeters above the plate. A potential difference of 125 volts or approximately 600 volts per centimeter was impressed across the terminals, one of which was grounded. A Dolazalek quadrant electrometer whose sensitivity was 10^{-14} amps per millimeter deflection was employed to measure the current. Ultra violet light was projected onto the oil from above from the iron-nickel arc described previously. In neither of these experiments was there any indication of any photo-electric effect which could be measured on our instruments. It was concluded that there was no appreciable photo-electric emission of the oil due to those wave lengths of ultra violet light transmitted by quartz.

D. THE DEPENDENCE OF RESISTIVITY UPON THE VITAMIN A CONTENT OF THE OIL

A sample of the oil was allowed to stand open to the air for nearly three months, which unquestionably reduced its vitamin A activity considerably. The changes in resistance of this oil showed no marked differences from those noted in the samples left open for four or five days. Also, the oil through which air at high temperature had been bubbled for sufficient time to destroy the vitamin A, and then allowed to stand for a few days, showed no appreciable change in resistance. In neither case was the change very greatly different from that in the case of the oil which had been kept tightly stoppered and in the dark and which was fairly high in vitamin A. It would appear from these data that there is no connection between the electrical resistivity and the vitamin A content of cod liver oil. It should be noted, however, that heating the oil to high temperatures, does cause a permanent reduction in the resistance.

E. MISCELLANEOUS

As there was a possibility that the change in resistances noted might be due to some reaction between the oil and the brass electrodes, new electrodes of both platinum and copper were tried; but the results obtained with these did not differ from those obtained with the brass electrodes.

A variation of the voltage across the cell from five volts to 120 volts in steps of five volts, failed to show any effect due to polarization. This is shown in figure 3(b).

SUMMARY OF RESULTS

It has been shown by these experiments that:

1. The specific resistance of cod liver oil at room temperature is of the order of 2×10^{11} ohm-cms., but this value depends upon the previous treatment of the oil. This value increases to about 2×10^{13} ohm-cms. at 0° C. and drops to about 2×10^{10} ohm-cms. at 90° C.
2. The resistance varies with the temperature, in all cases decreasing with increase of temperature. There is a logarithmic relation between resistance and temperature over a considerable range in temperature. The changes in slope of the logarithmic graphs do not always occur at the same temperature and are not always in the same direction. The resistance becomes very unstable at temperatures between 80° C. and 90° C. and is permanently decreased when heated to those high temperatures. There is, however, no permanent change due to lowering the temperature to -5° C.
3. The resistance of the oil depends upon the exposure to air decreasing with slight aeration, then upon further aeration increasing to as much as three or four times its original value and eventually decreasing again to its original value or even slightly below this.
4. There is no change in the conductivity of the oil when exposed to ultra violet light of such wave lengths as are passed by quartz.
5. There is no definite relation between the resistance of the oil and its vitamin content.
6. The resistance does not depend on the voltage impressed across the cell; that is, there is no polarization effect.
7. The change in resistance is not due to any chemical action between the oil and the electrodes.

METHODS FOR THE DETECTION OF LIPOLYSIS BY MICROORGANISMS¹

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The ability of a microorganism to hydrolyze fat is of significance in certain changes occurring in food products and also in the classification and identification of species. This character has received relatively little attention; one of the reasons is that methods for its detection have developed more slowly than those revealing changes in carbohydrates and proteins.

Various procedures for the detection of lipolysis by organisms have been suggested. A number of these have been compared at the Iowa Agricultural Experiment Station, particularly in connection with investigations on the lipolytic organisms in dairy products. The detailed methods that have been found most useful are presented herein.

THE NATURAL FAT TECHNIC

In the natural fat technic lipolysis is detected by a change in the appearance of small globules of fat distributed through a medium. Eijkman (7) apparently first noted this change. He poured melted agar over a thin layer of tallow in the bottom of a petri dish and found that the fat under lipolytic colonies became white and more opaque.

A convenient procedure for the natural fat technic is to add a small amount of fat emulsion to each plate before pouring with agar; beef infusion agar is very satisfactory, although various media can be used. The proper fat distribution is readily obtained by preparing a fat emulsion as follows: 3 ml. of a natural fat, 0.5 gm. of agar, and 100 ml. of water are sterilized in a 6-oz., screw cap bottle; after cooling, the fat is dispersed as fine globules by very vigorous agitation. Approximately 0.5 ml. of this emulsion is used per plate. In the case of a high melting point fat it may be necessary to rewarm the mixture before agitation in order to properly disperse it.

Around actively lipolytic colonies the fat globules become opaque through the action of the organisms (plate I). The change in the appearance of the fat globules apparently is due to partial lipolysis since when the plates are flooded with a suitable indicator, free fatty acids are detected. Probably some of these acids are of relatively high molecular weights and, being insoluble in the agar, remain in the fat globules and account for the changed appearance. Various fats can be used in this technic. With those of higher melting points (for example, butter fat) it is necessary to warm the plates in a 45° C. incubator prior to examination since at room temperature the fats are largely solid and rather opaque.

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With low melting point fats, such as cottonseed oil or corn oil, warming is not necessary because the fats are liquid at room temperature.

In plating various materials for lipolytic organisms, or when plates are spotted with cultures to be tested for lipolysis, the accuracy of the results obtained with the natural fat technic can be confirmed by other methods. Sometimes it is impossible to detect weakly lipolytic colonies by the technic. Hastings (9) employed essentially this procedure in studying lipolysis but used more fat in the medium and, by pouring the plates with hot agar, allowed the fat to come to the surface.

The natural fat technic does not interfere to any extent with the determination of total numbers of organisms and it does not involve flooding the plates with a dye so that colonies are easily picked. The inability to detect weakly lipolytic colonies may at times be a serious objection.

THE NILE BLUE SULFATE TECHNIC

Nile blue sulfate has long been used in histology as a fat stain and recently has been employed as an indicator of lipolysis because of its ability to color neutral fats pink and fatty acids blue. As noted by Rettie (13), nile blue sulfate consists of two stains, the oxazine form and the oxazone form. The latter is soluble in fats and fat solvents, giving a pink solution. Thorpe (17) studied the action of nile blue sulfate in the staining of fat and found that while the red dye was not originally present in the solid dye it was apparently formed upon dissolving in water. The amount of red dye could be increased by heating nile blue sulfate in a weak sulfuric acid solution. Nile blue sulfate is the sulfate of diethylaminophenonaphthoxazine and the red dye the corresponding oxazone.

Smith (15) showed that in the presence of nile blue sulfate globules of olein or other neutral fats were red while those of oleic acid were blue, the blue color resulting from the formation of a soap between the fatty acid and oxazine base. The ability of nile blue sulfate to color the various triglycerides and fatty acids was investigated by Boeminghaus (3). Oleic acid gave an intense color compared to various other fatty acids. Kaufmann and Lehmann (11) noted that the unsaturated fatty acids and unsaturated triglycerides stained especially well with nile blue sulfate although certain other saturated compounds also were well stained. Collins and Hammer (5) found that while higher saturated fatty acids and triglycerides did not color well with nile blue sulfate the lower saturated compounds were well colored. Oleic acid and triolein were also well stained.

Turner (18) used nile blue sulfate as an indicator of bacterial lipolysis by adding the dye to a medium containing dispersed fat. Fat globules in the vicinity of lipolytic colonies were stained blue while those at a distance were stained pink. The same investigator (19) compared various methods for the detection of lipolysis and found that the nile blue sulfate medium was very sensitive and gave excellent differentiation; however, the dye was inhibitory for certain species of organisms. Hussong (10), in studying lipolysis by organisms, used a modification of the Turner technic. Collins and Hammer (5, 6) and Hammer and Collins (8) determined lipolysis by a technic similar to that of Hussong.

A convenient procedure for the nile blue sulfate technic is to add to each plate 0.5 ml. of a fat emulsion, such as the one used in the natural

fat technic, and pour with beef infusion agar containing nile blue sulfate in the proportion of 1 part of the dye to 10,000 parts of agar. Generally the dye (5 ml. of a sterile 0.2 per cent aqueous solution per 95 ml. of agar) is added to the agar just before pouring the plates, rather than before sterilization, since with the former method a more desirable color is imparted to the medium. Lower concentrations of the dye can also be used.

Examination of the plates for lipolysis is greatly aided by the use of a hand lens or low power binocular. The unhydrolyzed fat is stained pink while with partial hydrolysis the fat globules become blue. The change is from a distinct pink to a distinct blue so there is no difficulty in detecting it. Around colonies of actively lipolytic species there may be a disappearance of the blue dye from the medium due to its absorption by the fatty acids. The method is very useful for studying lipolysis by organisms spotted on plates and can be employed also in plating for numbers of organisms. Since the dye is added to the agar, the picking of desired organisms is not complicated by flooding the plates. The primary disadvantage of the technic is that certain organisms, especially streptococci and micrococci, are inhibited by the dye. In some instances this may be an advantage because of the fact that the medium has a selective action. Thus it is sometimes possible to isolate lipolytic organisms when they constitute only a small percentage of the total flora; it should be emphasized, however, that nile blue sulfate may inhibit both non-lipolytic and lipolytic species.

In order to avoid inhibition of organisms by nile blue sulfate, Turner's technic has been modified (16) by first staining the fat pink with a concentrated solution of the dye and then washing it free of the excess stain. Organisms are grown on a solid medium containing the stained fat in a dispersed state and lipolysis is detected by a change in the color of the fat globules from pink to blue. In attempts to employ this method the fat globules in the vicinity of lipolytic bacteria did not become blue. There was a change in the appearance of the globules which is probably related to the change occurring in the natural fat technic. Since the oxazine form of nile blue sulfate, which reacts with the fatty acids (13, 17), was entirely washed away, no typical blue color on hydrolysis would be expected.

The nile blue sulfate technic is a simple one and the results are easily read. The toxicity of the dye, however, does not permit satisfactory total counts and inhibits certain lipolytic species.

THE MODIFIED NILE BLUE SULFATE TECHNIC

Compounds other than nile blue sulfate have been used to detect the action of bacteria on fat. Sayer, Rahn and Farrand (14) detected lipolysis in a sugar free, litmus agar by a change in the color of litmus, while Waksman and Davidson (20) used various acid indicators for its detection; the fatty acids liberated by lipolytic organisms produced a change in the colors of the indicators.

Carnot and Mauban (4) and later Berry (2) detected lipolytic organisms by growing them on a solid medium containing dispersed fat and, after incubation, flooding the plates with a saturated aqueous solution of copper sulfate. The free fatty acids united with the copper sulfate to form an insoluble blue copper soap which was readily discernible.

Long and Hammer (12) detected lipolysis by flooding plates with nile blue sulfate solution rather than copper sulfate solution.

In the modified nile blue sulfate technic organisms are grown on plates containing a dispersed natural fat; 0.5 ml. of a fat emulsion, prepared in the manner described, is added to each plate and after incubation the plates are flooded for 30 minutes with aqueous nile blue sulfate of an approximate strength of 1 to 1500, rinsed with water, and observed for lipolysis. After rinsing it is an advantage to hold the plates several hours at 45° C. in order to intensify the staining. The unhydrolyzed fat globules are colored pink while partly hydrolyzed globules are blue. Nile blue sulfate is used, rather than copper sulfate, because it gives a sharper differentiation. However, nile blue sulfate stains the medium more or less deeply while copper sulfate does not.

In many trials with various dairy products the total counts obtained with the modified technic were always considerably higher than those obtained with the nile blue sulfate technic ordinarily employed. The lipolytic counts were much higher also with the modified technic. The flooding of the plates after incubation is a disadvantage, however, in that the picking of colonies is complicated. This difficulty can be overcome by special purification of the cultures picked from plates or by pouring a second set of plates for isolation purposes.

The modified nile blue sulfate technic is especially useful since it avoids the inhibitory effect of the dye and is very satisfactory for both total and lipolytic counts. The results appear to be reliable and are easily observed. The chief defect is that the picking of colonies is complicated by flooding the plates.

THE SIMPLE TRIGLYCERIDE TECHNIC

Anderson (1) proposed a technic for detecting lipolysis in which the organisms are grown on a solid medium containing dispersed tributyrin. Since the products of hydrolysis of this compound are soluble, lipolysis can be detected by a disappearance of the globules around lipolytic colonies. Collins and Hammer (5) studied the action of bacteria on simple triglycerides, using nile blue sulfate in the media. They noted that with certain of the simple triglycerides the globules often disappeared because of the solubility of the products of hydrolysis.

The simple triglyceride technic can be carried out conveniently by preparing an emulsion of tripropionin or tributyrin in 0.5 per cent agar and adding some of this to the plates before pouring. Another procedure is to add a few drops of the triglyceride to a tube of melted agar, disperse it by vigorous shaking, and pour the mixture into a petri dish. The amount of triglyceride, whether used as an emulsion or directly, varies with the solubility of the compound. In the preparation of an emulsion, from 4 to 6 per cent tripropionin or tributyrin is satisfactory. Triacetin cannot be used because of its relatively high solubility and triglycerides higher in the series than tributyrin are unsatisfactory because, in the event of hydrolysis, the fatty acids liberated are only slightly soluble in the agar. After the incubation of poured plates, or of plates on which cultures have been spotted, there is a disappearance of the triglyceride globules around colonies of actively lipolytic species. In order to obtain clear cut results it is necessary to have the triglyceride evenly distrib-

uted in small globules and the layer of agar comparatively thin. With large globules the time required for hydrolysis is too long and if there are not enough globules the action is not readily observed. When the agar layer is too thick it may be impossible for a colony to hydrolyze the globules through the entire depth of the layer.

While bacteria capable of hydrolyzing natural fats will produce a clear zone around the colonies when inoculated on a plate containing dispersed tripropionin or tributyrin, certain cultures commonly regarded as non-lipolytic, also will bring about this reaction. Long and Hammer (12) found that certain cultures of *Streptococcus liquefaciens* hydrolyzed tripropionin, tributyrin, or both, but gave no evidence of hydrolyzing either cottonseed oil or butter fat. Recently 32 *Streptococcus lactis* cultures of various origins were studied. None of them gave any evidence of attacking cottonseed oil. Tripropionin was easily hydrolyzed, only two of the cultures failing to attack this compound. Tributyrin was less easily hydrolyzed by *S. lactis* than tripropionin, since only 17 of the 32 cultures tested gave a positive reaction. This agrees with the findings of Collins and Hammer (5) who noted that hydrolysis of the simple triglycerides of the saturated fatty acids became more difficult as the molecular weight increased.

In general, the observations on the simple triglyceride technic indicate that this method is not a satisfactory means of selecting organisms capable of hydrolyzing natural fats because the lower triglycerides are much more easily hydrolyzed than natural fats.

DETECTION OF PROTEOLYTIC AND LIPOLYTIC ORGANISMS

Many of the lipolytic organisms are also proteolytic and in some instances it is an advantage to study proteolysis and lipolysis on one plate. The various technics used for the determination of lipolysis can be modified by the addition of skim milk, a procedure that has long been used to detect proteolysis. This may be conveniently carried out by adding 0.5 ml. of sterile skim milk to each plate and is especially satisfactory in connection with the modified Nile blue sulfate technic. The procedure is less useful with the natural fat technic because lipolysis may be difficult to detect when there is no clearing, due to proteolysis, around a colony. With either procedure a count of the acid forming organisms can be obtained in addition to the total, proteolytic and lipolytic counts if sugar enough is present to yield the amount of acid needed to precipitate the casein around a colony. This may require the addition of lactose or glucose to the medium. With certain acid forming species the rapid acid production in the presence of considerable sugar may interfere with the growth of various organisms on the plates.

SUMMARY

Various methods can be used to study the ability of an organism to hydrolyze fat dispersed in a medium. In the natural fat technic hydrolysis is detected by a change in the appearance of globules of fat which become more opaque; with this method it is difficult to detect weakly lipolytic organisms. In the Nile blue sulfate technic the dye is added to the medium and lipolysis is readily noted by a change in color of the fat

globules from pink to blue. This technic gives especially clear cut results but the toxicity of the dye definitely limits its usefulness. In the modified Nile blue sulfate technic lipolysis is readily detected after incubating the plates by flooding them with a solution of Nile blue sulfate; unhydrolyzed fat is pink while partly hydrolyzed fat is blue. The flooding of the plates complicates the picking of colonies.

If globules of tripropionin or tributyrin are dispersed in the medium in place of a natural fat there is a disappearance of the globules in the vicinity of lipolytic colonies because of the solubility of the products of hydrolysis. Since these triglycerides are easily hydrolyzed, a positive test is not always an indication of ability to hydrolyze natural fats.

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PLATE I

Changes in globules of cottonseed oil partly hydrolyzed by *Achromobacter lipolyticum*. The globules around the spot colonies are white and opaque.

PLATE I



THE LIBRIFORM FIBERS IN THE ROOTS OF SWEET CLOVER, MELILOTUS ALBA DESR.

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The libriform fibers, common xylem structures of Angiosperms and so named by Sanio (12) because of their resemblance to the fibers of the bast (formerly called liber), are slender spindle-shaped fibers with walls sparsely pitted and commonly so much thickened that the cell cavity is almost obliterated. Their walls, in common with those of most wood and bast fibers, have been described generally as consisting of three rather easily distinguishable and separable layers. According to the recent terminology used by Kerr and Bailey (4) and Anderson (2), who regard the outermost (middle lamella) of the three layers as an intercellular deposit, the wall of each individual fiber consists of two layers, of which the outer is designated as the primary and the inner as the secondary layer. A close study usually reveals that the secondary and commonly the primary layer are not unit structures but are composed of delicate concentric lamellae that closely fit over and tightly adhere to each other. It is the secondary layer that varies greatly in thickness and when fully developed, commonly constitutes as much as three-fourths or more of the cross sectional area of the fiber.

The layers of the walls of libriform fibers differ from the middle lamella and from each other in both chemical and physical properties. The secondary layer, which has received most consideration because of its functioning apparently as reserve food material, has been variously reported as consisting of hemicellulose, cellulose, and lignin. It is usually pliant, resembling a stiff mucilaginous or gum-like substance, and separates readily from the much more rigid and commonly more lignified primary layer.

The removal of the secondary layer during spring growth, a phenomenon indicating that this layer is reserve food, has been observed a number of times, notably in the roots of the pear, peach, quince, raspberry, and willow, by Le Clerc Du Sablon (5), in the stems of the grape and black locust by Potter (8), and in the stems of the alder, birch, horse-chestnut, ash, and some Gymnosperms by Schellenberg (13). During the process of dissolution, Schellenberg observed that the secondary layer became ramified by canals, a feature suggestive of the corrosive action of diastase on starch grains. In the wood of fruit trees, especially in apple trees, as shown by Murneek (7), Roberts (10), and others, there are fibers of the libriform type with secondary layers of reserve carbohydrates. Murneek refers to these reserve carbohydrates in the walls as hemicellulose.

A survey of the literature shows that the libriform fibers are especially prominent in the stems of the legumes. Sanio (11, 12) found libriform tissue well developed in a large number of the Papilionaceae, among which were *Medicago arborea*, *Cytisus laburnum*, *Gleditsia triacanthos*,

and *Caragana arborescens*. In the stems of *Robinia pseudo-acacia* and of species of *Wistaria*, Strasburger (14) observed that the fibers of the xylem had a much thickened inner layer which reacted to tests for cellulose. Potter (8) noted libriform fibers in the stems of *Vicia faba*, and in the roots of some species of *Lupinus* and *Phaseolus*.

In all libriform fibers investigated by Potter the secondary layer responded to tests for cellulose. In the roots of *Trifolium repens*, the secondary layer of the fiber walls according to Erith (3), is pliant and becomes red in the presence of chlor-zinc-iodide. Sanio (11) found the secondary layer in the walls of the libriform fibers of the legumes commonly gelatinous and was of the opinion that libriform fibers with gelatinous secondary layers are characteristic of the Papilionaceae.

MATERIAL AND METHODS

The investigation centered mainly on the primary and secondary layers of the libriform fibers, only casual attention being given to the middle lamella since in this investigation it was found to have no features of particular significance. The investigations included the structure, chemical constitution, and physical properties of the walls of the fibers, and the modifications the walls undergo during the second season's growth.

The libriform fibers investigated were chiefly those in the roots of the common strain of biennial white sweet clover. The observations on other biennial strains, including the biennial yellow sweet clover, were sufficient to show that the biennial types are very similar in the structure and history of their libriform fibers. In the roots of the annual white sweet clover, although the libriform fibers are similar in structure and properties to those of the biennial types, they are relatively few, their formation being limited to the early period of growth and apparently have no important function in the life of the plant aside from the rôle they play in the early contraction of the root and hypocotyl. Roots of the biennials in the first season's growth furnished fibers in various stages of development, and thus suitable for structural, physical, and chemical studies of fiber development, while roots in the second year's growth afforded fibers for the study of the modifications their walls undergo the second season.

The conclusions concerning the chemical nature and physical properties of the libriform fibers were based largely upon their reactions observed under the microscope. The sections were made mostly free-hand and from fresh material. Some microtome sections of imbedded material were employed in studying the minute structure of the fiber walls and for making some of the photomicrographs.

The microchemical studies of the fiber walls were based upon their color reactions, and solubility. In identifying the substances by means of positive color reactions the reagents used were as follows: Ruthenium red and methylene blue for pectins; phloroglucin and HCl for lignin; and chlor-zinc-iodide and iodine with H_2SO_4 for cellulose. Safranin and hematoxylin, which were used in making permanent mounts, also served in the recognition of cellulose and lignin.

The use of the solubilities of substances as an aid in their identification involved also the color reactions of the substances. It was based upon the comparative color reactions of fibers treated and untreated with the

solvents of the respective substances, whose identifications were in question. In case of pectin which ruthenium red and methylene blue had indicated was present in the fiber wall, the color tests were repeated on fibers that had been boiled in ammonium oxalate or in weak H_2SO_4 for three or four hours and then washed in water. In the identification of lignin by its solubility fibers which had been boiled in ether-alcohol, then in one per cent NaOH, chlorinated, and then boiled in two per cent sodium sulphite to remove the lignin, were compared with fibers not previously treated as to color reactions for lignin. In the identification of hemi-cellulose and cellulose by their solubilities both color reactions and changes in thickness of wall layers of fibers previously treated with Schweitzer's reagent or with various concentrations of H_2SO_4 were considered in the comparisons.

In the study of such features as length, surface markings, shape, and structure of the fibers that required lengthwise views, long longitudinal sections were used, which after being boiled in one per cent NaOH, in Schultz's maceration fluid, or in five or ten per cent H_2SO_4 , were separated into their individual fibers. In studying the lamellae and fibrillar structure of the wall layers, the fibers were swollen in high concentrations of H_2SO_4 (60 per cent or higher) in the presence of iodine. The swelling of the fibers was followed under the microscope and controlled by varying the concentration of the acid or by displacing it when the layers were satisfactorily swollen.

In sweet clover plants beyond the seedling stage the hypocotyl and radicle, readily distinguishable in the seedling stage, become so fused and similar histologically that it is not necessary to refer to the hypocotyl separately from the root system. The root system consists of a large commonly branched taproot and relatively small lateral roots. In the biennial sweet clovers the root system approximates its maximum size and the libriform fibers reach their maximum number and thickness of walls the first season. During the second season the changes in the tissues of the root have to do almost entirely with the removal of storage materials and cell wall modifications.

THE LIBRIFORM FIBERS OF SWEET CLOVER DURING THE FIRST SEASON

GENERAL FEATURES

In the roots of the biennial sweet clover, the libriform fibers are quite variable in different plants, but constitute at the end of the first season's growth, from a fourth to more than one-half of the volume of the xylem (figs. 1 and 2). As shown in cross-sectional views of the roots, the xylem is divided radially by rays into wedge-shaped masses which consist largely of libriform fibers arranged in groups of various sizes (fig. 1). Interspersed among and alternating with the groups of libriform fibers are the tracheal elements and some parenchyma. The libriform fibers are typically spindle-shaped with a range in length from one to three or more millimeters and an average width of approximately seven microns at their widest place. The individual fibers of the fiber bundles are not only joined laterally but also lengthwise by their overlapping ends, and thus constitute tough flexible strands that transverse the entire length of the root. Throughout the length of the fiber bundles there are included here and there small isolated groups of parenchyma cells. By the development

and expansion of these isolated parenchyma tissues the fiber strands are partially separated into smaller anastomosing strands with sinuous courses, the result being that the system of fiber strands is shortened and the root and hypocotyl thereby lengthwise contracted and pulled farther into the soil where they have better protection against unfavorable weather conditions.

STRUCTURE OF THE LIBRIFORM FIBERS

In fully developed libriform fibers of the sweet clover, like those characteristic of roots at the end of the first season, the two layers, primary and secondary, are readily recognizable (fig. 9). The middle lamella is thin and appears in untreated sections as scarcely more than a line of junction between the opposite primary layers. The primary layer is relatively thin as compared with a fully developed secondary layer but its thickness is a number of times that of the middle lamella. Its maximum thickness is attained early in the fiber's development, is practically the same in all fibers, and is uniformly maintained throughout the first season and during the second season until near the flowering period of the plant (fig. 3 and 5). The secondary layer, which, unlike the primary layer, is quite variable in thickness, commonly is 75 per cent or more of the cross-sectional area of the fiber wall, and so much thickened that the cavity of the fiber is almost obliterated and the protoplasm reduced to an almost undetectable quantity (fig. 5).

In fibers suitably swollen and stained, a fibrillar structure is revealed in both wall layers and also in the middle lamella. The primary layer consists of a single lamella, one layer of fibril strands in thickness. The fibril strands are arranged spirally with the coils almost at right angles to the long axis of the fiber and so closely joined that they are visible only when forced apart by the swelling of the fibers. The fibrils of the primary layer are oriented about those of the secondary layer much after the fashion of the cord about a fagot, and when the secondary layer expands lengthwise in swelling, the coils of the fibrils of the primary layer are separated sufficiently to become recognizable.

In secondary layers fully thickened, a number of lamellae, sometimes as many as 10, can be recognized when the layer is suitably swollen and stained (fig. 6). The thickness of this layer depends upon growth conditions. In libriform fibers in the process of development, as in those near the cambium during the growing season and in old fibers where storage materials are being removed, there is a wide range in the thickness of the secondary layer. The thickness of this layer depends also upon the nutritional conditions of the plant. Frequent mowing and close pasturing, which hinder the manufacture and deposition of carbohydrates, affect not only the number of libriform fibers formed but also the thickness of their secondary layer.

The secondary layer is pliant like rubber or a stiff gelatinous material and is commonly distorted by folding inward when the size of the lumen permits, which is most likely because of its inability to expand in the direction of the primary layer. Some of the folding of this layer so frequently observed in cross sections may be due also to the pressure of the knife in sectioning (fig. 4). The ease with which it is separated from the more rigid primary layer indicates that the two layers are joined very loosely, if joined at all. The secondary layer, although rather resistant,

swells much more readily in hydrating agents than either the primary layer or middle lamella (fig. 9). In high concentrations of swelling agents, as 60 per cent H_2SO_4 or in Schweitzer's reagent, the secondary layer swells and bursts through the more resistant primary layer at intervals along the surface of the fiber and expands into large, rounded swellings, which often cause the fiber to resemble a chain of beads (fig. 7). In cases of short sections of fibers exposed to high concentrations of the hydrating agents, the secondary layer expands lengthwise beyond the ends of the primary layer and forms large, rounded swellings that give the sections the resemblance of dumb-bells, and this again clearly demonstrates its greater response to the hydrating reagents (figs. 8, 9, and 10).

Each of the component lamellae of the secondary layer is composed of strands of fibrils which in all the lamellae are parallel with the long axis of the fiber and almost at right angles to those of the primary layer (fig. 8). The fibrils are very minute and their detection is difficult unless the layer is partially hydrated in the presence of iodine in which the fibrils become somewhat colored.

The wall layers of the libriform fibers of sweet clover are like those of wood and bast fibers as described by Ritter (9), Ludtke (6), and Anderson (1), in that the lamellae are composed of fibrillar strands, the chief features of distinction being in the relative orientation of the fibril strands in the different layers and lamellae.

REACTIONS OF THE WALLS OF THE LIBRIFORM FIBERS TO HISTOLOGICAL STAINS, MICROCHEMICAL COLOR REAGENTS, AND SOLVENTS

In fibers stained with different stains, as safranin and hematoxylin, the primary layer shows some affinity and the secondary a decided affinity for the cellulose stains. The decided cellulose nature of the secondary layer is also well shown by its swelling and turning blue in the presence of such reagents as chlor-zinc-iodide, and combinations of H_2SO_4 and iodine. The primary layer, besides showing no great affinity for cellulose stains, behaves much like the middle lamella in reacting slowly and sometimes not at all in old fibers when treated with Schweizer's and other hydrating agents. In tests for lignin and pectin-like substances with phloroglucin followed by HCl and with ruthenium red or methylene blue, the reaction was strong in the middle lamella, especially in old fibers, a little less pronounced in the primary layer and weak to almost not detectable at all in the secondary layer.

Fibers boiled an hour in five per cent H_2SO_4 or HCl showed no change in thickness in either of the layers or in the reactions to the various tests. Boiled for an hour in one per cent NaOH or in chloral hydrate the fibers no longer reacted to the tests for pectin, but separated readily and still reacted to the cellulose and lignin tests. After being boiled in 10 per cent NaOH , fibers usually reacted to neither the pectin nor lignin tests, but to tests for cellulose, the primary layer now turning blue in chlor-zinc-iodide almost immediately while the secondary layer changed from a brownish to a blue after several minutes, but neither layer showed any reduction in thickness. Moreover, after the process for the removal of the lignin by boiling in ether-alcohol, then in one per cent NaOH followed by chlorination and boiling in two per cent Na_2SO_4 , both layers of the fiber walls showed no reduction in thickness

and the primary layer reacted promptly to the cellulose tests. In Schweitzer's reagent for two days, the secondary layer was much swollen, if not dissolved, but the primary layer was not much affected unless previously subjected to the process for the removal of lignin.

From the preceding reactions of the libriform fibers the following conclusions were drawn. The primary layer consists of cellulose impregnated with other substances, chiefly lignin. The secondary layer is either cellulose or a very resistant type of hemi-cellulose. That the layers maintain their thickness upon the removal of impregnated substances is in accord with the present conception that the secondary materials in cell walls are between the cellulose micellae and do not disturb their arrangement. The middle lamella in the libriform fibers of sweet clover apparently has its usual composition, consisting principally of lignin and calcium pectate.

Attempts to entirely dissolve the primary and secondary layers showed them remarkably resistant to both alkalies and acids, although the resistance was noted to vary considerably with the age and stage of the development of the fibers. Boiling them for 30 minutes in 40 per cent NaOH caused much swelling but did not dissolve either of the layers. In the autoclave at two atmospheres of pressure for an hour the secondary layer disappeared usually in 30 to 35 per cent H_2SO_4 while the primary layer usually endured concentrations up to 40 per cent of the acid. The resistance to hydrolysis of the primary layer can be attributed to the presence of lignin. In resistance to hydrolysis the secondary layer is like ordinary cellulose but in functioning as a wall reserve it has the nature of hemicellulose. It is evident that methods of chemical analysis in which weak hydrolyzing agents are used do not include the carbohydrates stored in the secondary layer. Roberts (10) has called attention to a similar situation in apple trees where the reserves in the form of wall thickenings were found to resist remarkably high concentrations of acids.

THE MODIFICATIONS OF THE LIBRIFORM FIBERS AND ACCOMPANYING TISSUES DURING THE SECOND SEASON

During the second season while the biennial sweet clover is developing its flowering shoots and maturing seeds, transformations occur in the tissues of the roots which consequently lose their flexibility and become woody and rigid. This may be interpreted as an adaptation which enables the plant to support its reproductive shoots in an erect position. The processes bringing about the change in texture has to do with the digestion of the secondary layer of the libriform fibers and the thickening and lignification of the primary layer of the fibers and also of the walls of other tissues. The progress of these processes is most rapid during the latter part of the season when the seeds are ripening.

The digestion of the secondary layer is first noticeable in the libriform fibers near the cambium. In some of the fibers the secondary layer is almost entirely removed while in others it is only reduced in thickness and left usually much folded and shrunken away from the primary layer. The digestion proceeds centripetally until nearly all the fibers have their secondary layer to some degree digested. This process of digestion is accompanied or soon followed by a decided increase in the thickness

of the primary layer and its strong lignification. At the same time the libriform fibers undergo their modifications there is a thickening and lignification of the walls of the ray cells and of other parenchyma, the process in these tissues beginning also in the region of the cambium and proceeding centripetally. These are the cell wall modifications that replace the fleshy flexible nature of the root during the first season by the woody rigid texture present at the end of the second season. The transformation culminates in the death of the root.

That the wall reserves are used mostly in making the transformations that occur within the root is indicated by the fact that their digestion and accompanying cell wall modification occur mostly after the new lateral roots and flowering shoots of the second season have reached their full size. The early disappearance of the starch from the roots suggests that it is depended upon for the development of the new lateral roots and for the initial development at least of the flowering shoots.

The digestion of the wall reserves begins as early as that of the starch but takes place much more slowly and is most pronounced near the end of the second season. The first noticeable anatomical changes in the interior of the root at the opening of the second season's growth are the transformation of some xylem parenchyma cells in the region of the cambium into tracheal vessels and the partial digestion of the secondary layer of some of the adjacent libriform fibers. This close association of the formation of vessels with the change in adjacent libriform fibers suggests that at the very start of the second season's growth the wall reserves are called upon to some extent to support the anatomical changes within the root. But their tardy removal suggests that the wall reserves are set aside in the main to support the anatomical changes in the root that culminate in its rigidity, an adaptation probably necessary to enable the root to properly support the flowering shoot and also to permit all the food made by the flowering shoots to be applied to the development of the reproductive structures.

SUMMARY

PERTAINING TO THE FIRST SEASON

The libriform fibers constitute from a fourth to more than one-half of the volume of the roots of the biennial sweet clovers.

With the middle lamella considered as intercellular, the layers constituting each fiber wall are two, and are designated as primary and secondary layers.

The secondary layer is quite variable in thickness and when fully developed is so extremely thickened that the cell lumen is a minute cavity with an almost undetectable amount of protoplasm. It normally is found upon close inspection to consist of a number of thin concentric layers or lamellae closely joined or cemented together and each composed of fibrillar strands oriented with their long axis parallel with that of the fiber. It reacts for cellulose, requires high concentrations of alkalis and acids to hydrolyze it, but it is digested by the plant enzymes and apparently used in the formation of tissue structures, in which capacity it plays the part of a wall reserve of the nature of hemicellulose.

The primary layer, one lamina in thickness, contains, in addition to its framework of cellulose, much lignin and is more resistant to hydrolyz-

ing agents than the secondary layer. After treatments to remove lignin it reacts typically for cellulose. Its fibrillar strands are spirally coiled with the turns of the spiral running almost at right angles to the long axis of the fiber and also to the long axis of the fibril strands of the secondary layer.

PERTAINING TO THE SECOND SEASON

During the second season the secondary layer of the libriform fibers is subjected to a process of digestion which begins near the cambium and proceeds centripetally until most of the fibers have been affected, some having their secondary layer considerably reduced in thickness, and others having lost it entirely or being left with only a thin folded remnant of it.

Associated with the digestion and removal of the secondary layer there occur other modifications in which the primary layer of the libriform fibers and also the walls of the xylem ray cells and other parenchyma cells are considerably thickened and strongly lignified. These processes culminate as the seeds are ripening and in the death of the root. As a result of these changes in cell walls the fleshy flexible nature characteristic of the root of the biennial sweet clovers during the first season is replaced by the woody rigid character possessed by the root at the end of the second season.

The libriform fibers, therefore, serve two purposes, namely, as storage places of carbohydrates in the form of a wall layer and later as strengthening fibers that give rigidity to the root when the flowers and seeds are being borne.

Owing to the highly resistant nature of the wall reserves of the libriform fibers they are not included in the common chemical methods of carbohydrate analyses.

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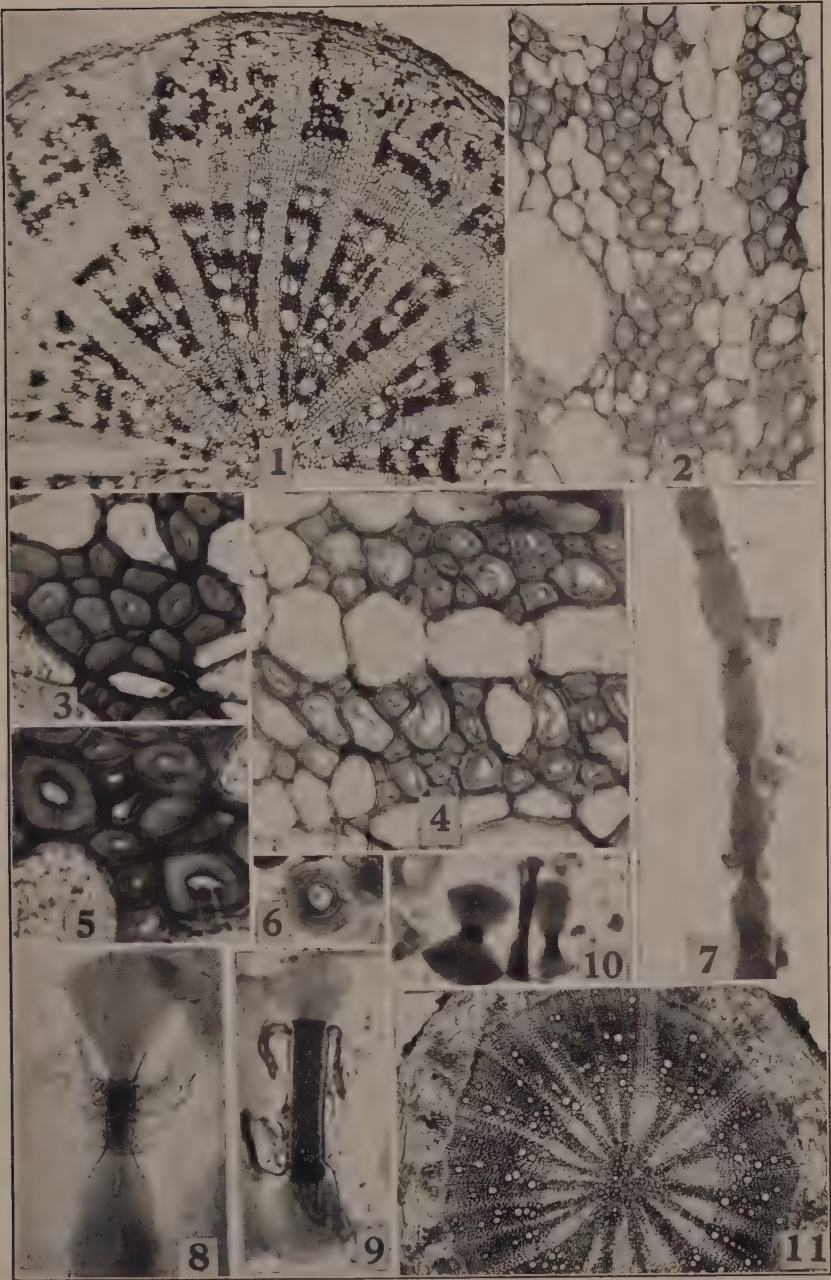
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EXPLANATION OF PLATE I

- Fig. 1. Cross section of a root showing relative amount of the libriform tissue,—the black areas in the xylem.
- Fig. 2. Portion of a cross section of a root in which the grouping of the libriform fibers is shown, and in which the amount of libriform tissue is proportionately very large, being three-fourths or more of the volume of the xylem.
- Fig. 3. Libriform fibers in cross section showing the primary and the extremely thickened secondary layer.
- Fig. 4. Cross sectional views of libriform fibers in which the secondary layer in some cases is folded and partially separated from the primary layer.
- Fig. 5. Cross sectional view of fibers in which some pits are visible.
- Fig. 6. Cross sectional view of a libriform fiber in which the lamellae of the wall layers are recognizable.
- Fig. 7. Lengthwise view of a libriform fiber much swollen in H_2SO_4 , showing the secondary layer broken through the primary except at the constrictions.
- Fig. 8. Lengthwise view of a much swollen section of a libriform fiber showing, (a) the fibrillar structure of the primary layer resembling a cord wrapped about the secondary layer, and (b) that the secondary layer consists of fibrils running parallel with the long axis of the fiber.
- Fig. 9. Lengthwise view of a section of a fiber in which the middle lamella, primary layer, and secondary layer are present.
- Fig. 10. Other lengthwise sections of fibers swollen in H_2SO_4 , showing a very great difference in the swellings of the primary and secondary layers.
- Fig. 11. Cross section of a root at the flowering stage of the plant, in which the transformations in the fiber and parenchyma tissues are well along. In the dark portion of the xylem, the libriform fibers have lost in part or entirely the secondary layer and the primary layer has been thickened and strongly lignified. The walls of the ray cells and the other parenchyma cells have been thickened and lignified.

PLATE I



FRACTIONATION OF OXIDIZED CORNSTALK LIGNIN^{1, 2}

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The hypohalogen oxidation of lignin for the purpose of recovering the oxidized product has in recent years received the attention of several workers (1, 4, 5). The reaction proceeds to a definite endpoint and the amount of oxidizing solution used can be quantitatively determined by means of sodium thiosulfate using starch paste as an indicator. Using an alkaline iodine oxidizing solution Walde (4) found that ammonia lignin from cornstalks required about 160 cc. of 0.1N iodine per gram sample. This oxidized lignin contained iodine and carboxyl groups, and was designated as iodo-carboxy-lignin.

Cornstalk ammonia lignin was prepared in this investigation. Oxidation values were determined for various conditions of oxidation, and the oxidized products were isolated. The various lignins were fractionated, methylated and saponified to obtain a more homogeneous product.

EXPERIMENTAL

PREPARATION OF CORNSTALK AMMONIA LIGNIN

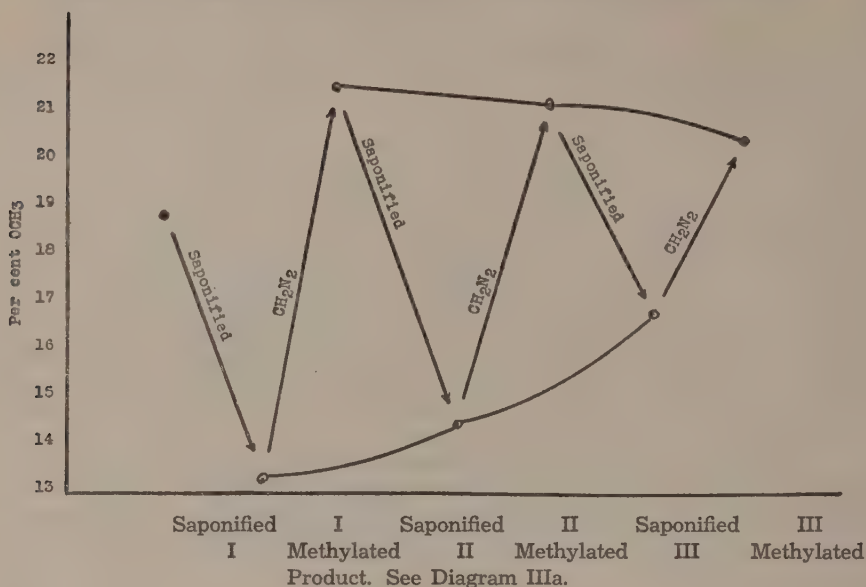
Since the various parts of the cornstalk contains approximately the same amount of lignin (2) the whole cornstalk was taken as a source of lignin. The stalks were allowed to air dry and were then put through a hammer mill. The ground stalks were subjected to an acid hydrolysis to remove the hemicellulose. The stalks were covered with 0.1N HCl and were hydrolyzed at a pressure of 20 pounds per square inch for two hours. The pulp remaining after this treatment was washed free of chlorides, and amounted to 62 percent of the original stalks, expressed on an air dry basis. The acid hydrolyzed stalks were placed in a steel bomb and covered with concentrated ammonium hydroxide. The bomb was closed and was heated by a steam jacket surrounding it so the pressure inside the bomb was 150 pounds per square inch. After six hours the bomb was allowed to cool and the insoluble pulp removed from the ammoniacal liquor by filtration. The ammoniacal liquor was heated to 50° C. and then acidified with concentrated HCl, which caused the lignin to precipitate. The lignin was allowed to settle overnight, the supernatant liquor decanted off and the remaining suspension filtered with suction. It was washed and dried, and on analysis was found to contain 5.14 per cent ash.

To remove the ash the product was dissolved in a dilute NaOH solution and then filtered with suction, first over macerated filter paper and then twice over filter paper. After warming, acidifying, filtering and

¹ A part of the thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of requirements for the Degree of Doctor of Philosophy.

² Supported in part by a grant from the Industrial Science Research funds of Iowa State College for the study of lignin.

Graph I. Change in methoxyl content by saponification and methylation.



washing as before, the product now contained but 0.37 per cent ash, and had a methoxyl content of 13.98 per cent.

OXIDATION VALUES

Alkaline iodine oxidation values were determined using a 0.2N iodine solution. The method consists essentially in dissolving a weighed sample of lignin in alkali, adding a slight excess of 0.2N iodine-potassium iodide solution, and after the oxidation is complete, acidifying and titrating the excess iodine with a standard solution of sodium thiosulfate. Cornstalk ammonia lignin gave oxidation values from 168 to 177 by the above method. An alkaline bromine oxidation gave values between 710 and 890, showing that the alkaline bromine oxidation proceeded much farther than the alkaline iodine oxidation. A neutral bromine oxidation using a sodium bicarbonate solution instead of an alkaline solution resulted in oxidation values of 201 to 203. From this it is seen that a neutral bromine oxidation oxidizes lignin to approximately the same degree as an alkaline iodine oxidation.

METHYLATION OF AMMONIA LIGNIN

Cornstalk ammonia lignin has a methoxyl content of 13.98 per cent and like other isolated lignins contains free hydroxyl groups which can be methylated. Using dimethyl sulfate and caustic according to the method of Urban (3) the methoxyl content was raised to 30.43 per cent. Methylation with diazomethane in an ether solution increased the methoxyl content of the original ammonia lignin to 24.43 per cent.

PREPARATION OF OXIDIZED LIGNIN

Cornstalk ammonia lignin was oxidized with a sodium hypobromite solution and the oxidized product recovered. The product obtained by this method was designated as bromo-carboxy-lignin II, abbreviated Br. C.L.II, to differentiate it from another bromo-carboxy-lignin prepared directly from the acid hydrolyzed stalks and which was designated as bromo-carboxy-lignin I, or Br.C.L.I.

For the preparation of bromo-carboxy-lignin II 30 grams of ammonia lignin were added to 1.5 liters of a solution containing 150 g. of Na_2CO_3 , being heated and stirred for several hours to insure complete solution. A stream of carbon dioxide was passed through the solution for four hours, causing much of the lignin to precipitate. A 10 cc. portion was tested for oxidation value to determine the exact amount of oxidizing reagent needed. The lignin solution was cooled to 6°C . and the calculated amount of an approximately 0.5N NaOBr solution was slowly added, the addition taking one-half hour. The oxidizing solution was made up in the ratio of 15 g. of Na_2CO_3 , 1 g. of NaOH, 20 cc. of bromine and sufficient water to make the volume to 1 liter. The calculated amount needed was 1,005 cc., so 1,200 cc. were added to maintain a slight excess. Ten minutes after the addition of the oxidizing solution the reaction was practically complete, as the excess oxidizing power was the same then as it was thirteen hours later. Since the quantity of lignin and the amount of oxidizing solution used by the reaction was known the oxidation value of the reaction could be calculated. This was 160

Diagram I. Dioxane separation of Br.C.L.I.

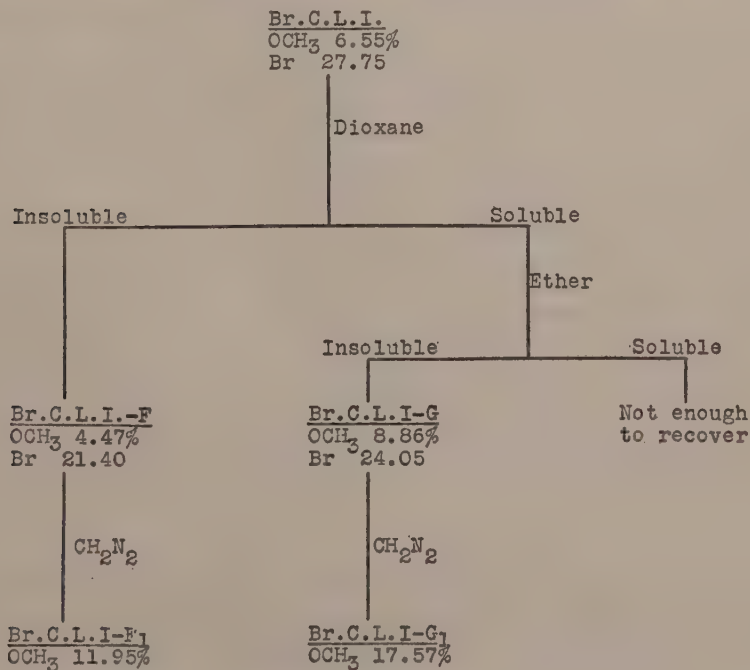
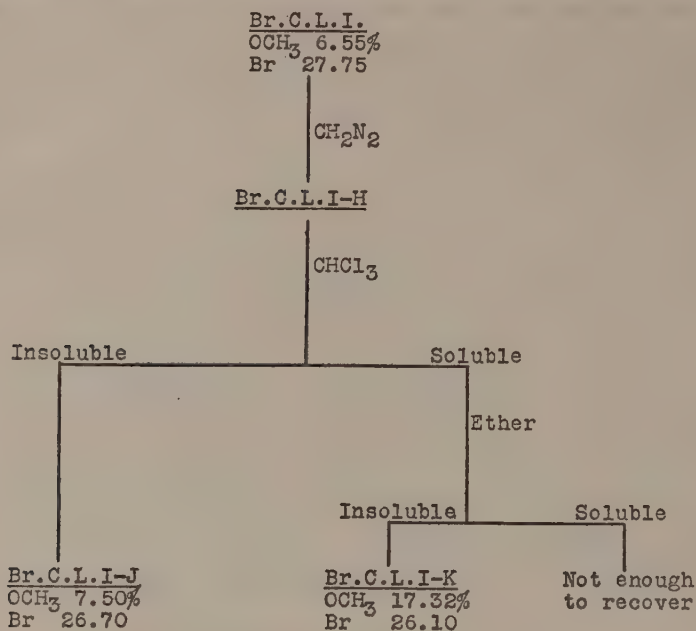


Diagram II. Chloroform separation of diazomethane methylated Br.C.L.I.



cc. of 0.1N bromine equivalent per gram, which is close to the alkaline iodine oxidation value.

The solution was acidified with concentrated HCl and allowed to stand overnight. The lignin had settled compactly in that time, the supernatant liquor was decanted and the lignin filtered, washed free of halogens and dried. The yield of air dry Br.C.L.II was 32 g., containing 5 per cent ash. This was dissolved in dilute caustic, filtered three times and again precipitated, washed free of halogens and dried.

A similar product was prepared directly from the acid hydrolyzed stalks, designated Br.C.L.I. In this case the acid hydrolyzed stalks were extracted with a dilute NaOH solution, filtered, and carbon dioxide passed in for 24 hours to saturate the solution. The calculated quantity of oxidizing solution plus a small excess was added. The solution was allowed to stand overnight, although the reaction was complete in 15 minutes. It was then filtered, acidified, and the precipitated oxidized lignin filtered, washed and dried. Analysis: Ash 5.3 per cent. The air dried sample was dissolved in dilute caustic, filtered three times, acidified, and the oxidized lignin filtered, washed and dried. Analysis: Ash 0.09 per cent, OCH₃ 6.55 per cent, Br 27.75 per cent.

METHYLATION OF OXIDIZED LIGNIN

Both bromo-carboxy-lignins prepared by the sodium bicarbonate method were subjected to methylation with diazomethane and dimethyl sulfate. Methylation was repeated in each case until the methoxyl content remained constant for two successive methylations.

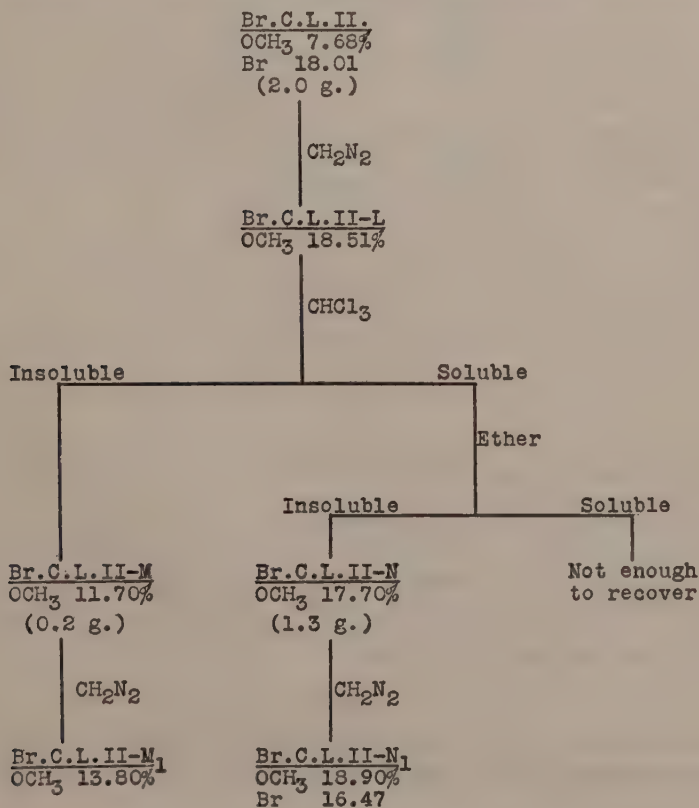
Br.C.L.I, with the analysis OCH_3 6.55 per cent, Br 27.75 per cent and ash 0.090 per cent was methylated with diazomethane in an ether solution three times. This methylated product had the analysis OCH_3 16.08 per cent, Br 26.78 per cent. The same Br.C.L.I, when methylated twice with alkali and dimethyl sulfate according to Urban's method, gave a product with the analysis OCH_3 16.80 per cent, Br 25.5 per cent.

Br.C.L.II, with the analysis OCH_3 7.68 per cent, Br 18.01 per cent and ash 0.0 per cent, after one methylation with diazomethane had the analysis OCH_3 17.87 per cent. The second methylation brought this value up to OCH_3 18.60 per cent, while the third checked the second at OCH_3 18.51 per cent, and had a bromine content of 16.70 per cent. With dimethyl sulfate and alkali this Br.C.L.II, after two methylations contained OCH_3 18.73 per cent, after the third methylation OCH_3 19.47 per cent, and after the fourth OCH_3 18.42 per cent, with a bromine content of 15.77 per cent.

FRACTIONATION

Br.C.L.I was separated into two fractions by means of dioxane. This is shown in Diagram I. The two products were methylated with diazo-

Diagram III. Chloroform separation of diazomethane methylated Br.C.L.II



methane and the methoxyl content of the two fractions after methylation were 11.95 per cent and 17.57 per cent for the insoluble and soluble fraction respectively.

Methylated Br.C.L.I can be separated into two fractions by means of chloroform and the soluble portion recovered by pouring into ether. This is shown in Diagram II. From an inspection of the methoxyl contents it would appear that Br.C.L.I-G₁ on Diagram I, which is soluble in dioxane, insoluble in ether and methylated with diazomethane, is very similar to Br.C.L.I-K on Diagram II, which is a diazomethane methylated product soluble in chloroform but insoluble in ether.

Diazomethane methylated Br.C.L.II can also be separated into two fractions by means of chloroform. This is shown in Diagram III. Br.C.L.II-N of Diagram III appears similar to Br.C.L.I-G₁ of Diagram I and Br.C.L.I-K of Diagram II from the methoxyl analysis, although it has a much lower bromine content.

SAPONIFICATION

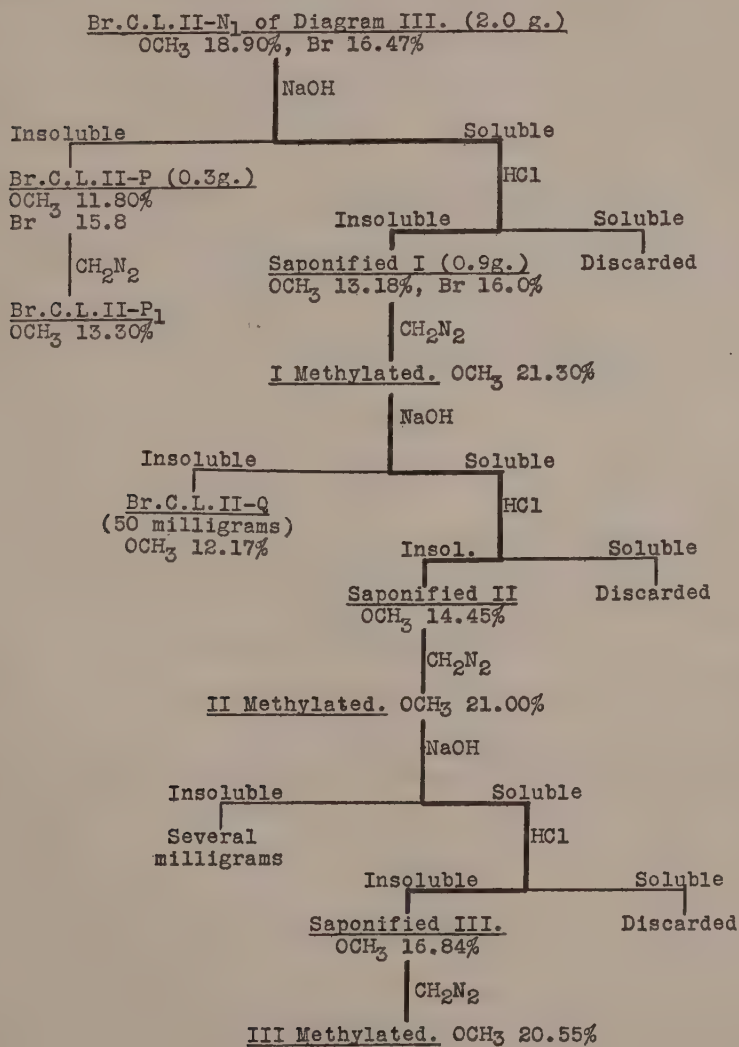
Br.C.L.II-N₁ of Diagram III, OCH₃ 18.90 per cent, was subjected to a saponification with 10 per cent NaOH for two hours. Not all the product dissolved by that treatment, so the insoluble portion, amounting to 15 per cent of the original and containing 11.80 per cent OCH₃, was filtered off. The filtrate was acidified, which precipitated the first saponified product, designated *Saponified I* and amounted to 45 per cent of the original Br.C.L.II-N₁. It contained 13.18 per cent OCH₃ and 16.0 per cent Br. This product was methylated three times with diazomethane, after which the methoxyl content was 21.30 per cent, as is shown in Diagram IIIa. This is considerably higher than the methylated Br.C.L.II-N₁ before saponification but since a 15 per cent residue containing only 11.80 per cent OCH₃ had been separated from it by saponification, this increased methoxyl content could be expected.

This *Saponified I*, diazomethane methylated product was designated *I Methylated* (see graph I), and was subjected to another saponification yielding *Saponified II* containing 14.45 per cent OCH₃, and an insoluble residue amounting to 50 milligrams and containing 12.17 per cent OCH₃. Methylation of *Saponified II* gave *II Methylated*, which contained 21.00 per cent OCH₃. This series is given diagrammatically in Diagram IIIa and the changes in the methoxyl contents are presented graphically in Graph I.

An inspection of Graph I shows that the methoxyl contents of the methylated products are all practically the same, about 21 per cent OCH₃. With the saponified products, however, an increasing methoxyl content is observed as the number of saponifications is increased. This shows clearly that something besides esterification and saponification is taking place, and indicates that repeated saponification and methylation would lead to a methylated product with about 21 per cent OCH₃ which would not saponify at all. Due to the small amount of product *III Methylated* the series was stopped at this point.

SUMMARY

1. Hypohalogen oxidation of ammonia lignin modifies the original hydroxyl groups in lignin so that diazomethane will increase the meth-

Diagram IIIa. Saponification and methylation of Br.C.L.II-N₁ of Diagram III.

oxyl content of the oxidized lignin as much as dimethyl sulfate. Ammonia lignin from cornstalks with 13.98 per cent OCH_3 with methylates to 24.43 per cent OCH_3 with diazomethane and to 30.43 per cent OCH_3 with dimethyl sulfate.

2. The presence of a carboxyl group in oxidized lignin is indicated by the fact that the methylated product saponifies with caustic and on remethylation the methoxyl content returns to the original value. However, the structure apparently is not stable in caustic, for on resaponification the methoxyl content does not drop as far as in the first saponification. With subsequent methylation and saponification the same effect is more pronounced.
3. Fractionation of bromo-carboxy-lignins with solvents by three different methods yielded apparently identical products with respect to the methoxyl content. Whether these products are homogenous or not is not clear, for the methylated products on treatment with caustic yield a soluble and insoluble fraction. However, the soluble fraction on methylation and saponification yields more insoluble material, indicating that the reactions involved are causing further changes in the lignin molecule.

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THE FERMENTATION OF XYLOSE BY THE COLON-AEROGENES GROUP OF BACTERIA¹

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The early investigations of Harden and associates (Harden 1901 et seq.) demonstrated fundamental differences between the two sections of the colon-aerogenes group. Many investigations have since been directed toward further elucidation of the mechanisms of dissimilation by which hexoses are converted into the various products. Consequently, studies generally have been limited to investigation of the fermentation of glucose or simpler compounds considered to be probable intermediates such as glyceric acid (Virtanen and Peltola, 1929; Antoniana, 1933), glyceraldehyde (Virtanen and Hausen, 1931), methylglyoxal (Neuberg and Gorr, 1925), pyruvic acid (Aubel, 1924), and dioxycetone (Virtanen, Karström and Turpeinen, 1929). The present investigation of the dissimilation of xylose has been carried out to contribute to our knowledge of the fermentation of pentoses by the colon-aerogenes bacteria.

EXPERIMENTAL

Comparative fermentations of xylose and glucose were carried out in liter flasks filled with medium and incubated at 30° C. for 7 days. With calcium carbonate as the buffer, the residual carbon dioxide in the liquor was determined in the following manner. After completion of the fermentation, the flask was connected to a reflux condenser leading to an ordinary soda-lime train for the gravimetric determination of carbon dioxide. A quantity of dilute (25%) sulphuric acid, slightly in excess of the total calcium carbonate originally added, was allowed to drop slowly into the flask and the contents slowly brought to boiling. The liberated carbon dioxide was transferred to the soda-lime tubes by drawing a slow stream of CO₂-free air through the apparatus for one hour. The initial CO₂ content was calculated from the accurately weighed CaCO₃ added. The difference between the quantity of CO₂ collected and that added as carbonate represents the CO₂ produced by the fermentation.

Other methods are described in previous papers. (Reynolds and Werkman, 1937, Reynolds, Jacobsson and Werkman, 1937.)

In all experiments the carbon and oxidation-reduction balances give evidence of the substantial accuracy of the analyses.

Identity of the cultures used was: *Escherichia coli*, number 26 of the American Type Culture Collection, and *Aerobacter indologenes*, strain described by Burkey (1928). Cultures were examined for purity microscopically and by plating both before and after completion of fermentation.

¹Supported by the Industrial Science Research Fund of Iowa State College.

Typical results obtained from glucose and xylose fermentations by *A. indologenes* (table 1) show that there are no great differences in the final products of the two sugars. Some points, however, are worthy of consideration.

Scheffer (1928) has formulated a scheme of dissimilation of glucose by *Aerobacter* (see also Kluyver, 1936). He proposed a primary cleavage to methylglyoxal. The intermediately formed methylglyoxal stabilized as lactic acid, or was either dehydrogenated to hydrogen and pyruvic acid or hydrolyzed to formic acid and hydrated-acetaldehyde. Acetylmethylcarbinol was formed by the condensation of one mole of the hydrated acetaldehyde with a mole of the non-hydrated, the latter arising through decarboxylation of pyruvic acid. Ethyl alcohol resulted from the reduction of intermediately formed aldehyde.

The data in table 1 on the fermentation of glucose can be shown to fit substantially the Scheffer-Kluyver scheme. In extension of their scheme Reynolds and Werkman (1937) and Reynolds, Jacobsson and Werkman (1937) have shown that acetic acid plays the rôle of an intermediary compound. The acid accumulates during the early phases of dissimilation and is later converted into 2,3-butylene glycol by reduction via acetylmethylcarbinol.

Scheffer attributed the formation of small quantities of succinic acid from glucose by *Aerobacter* to the protein (peptone) in his substrate. In agreement with such a conception, we have found that the fermentation of glucose by *A. indologenes* in a medium containing only ammonium salts as a nitrogen source yields no succinic acid. Under similar conditions, however, xylose gives rise to appreciable quantities of succinic acid. The acid was isolated and identified by its melting point and that of the paratoluidide. The finding of succinic acid as a product of fermentation of xylose by *A. indologenes* is in agreement with Fred and Peterson (1920) and Breden and Fulmer (1931). An explanation of the formation of the 4-carbon acid from xylose and not from glucose is not apparent.

In connection with the rôle of succinic acid in fermentation by *Aerobacter*, it has previously been shown that *A. indologenes* causes a decrease in that acid when its sodium salt is added to a glucose fermentation. (Reynolds, Jacobsson and Werkman, 1937). Disappearance of succinic acid was accompanied by increases in 1-carbon compounds and in 2,3-butylene glycol. The results suggested that the acid was broken down according to the Thunberg-Wieland series (Neuberg and Simon, 1933) yielding acetaldehyde which condensed to acetylmethylcarbinol, the latter being subsequently reduced to glycol. It is not impossible, that in the fermentation of glucose by *Aerobacter*, succinic acid is a normal intermediate which, like acetaldehyde, does not ordinarily accumulate. On the other hand, the presence of succinic acid as a product of the fermentation of xylose may be due to the properties of the particular type of fragment resulting from initial cleavage of the pentose molecule, possibly the 2-carbon fraction, assuming the initial cleavage is to 3- and 2-carbon compounds.

Results obtained from simultaneous fermentations of xylose and glucose by *Esch. coli* are collected in table 2.

TABLE 1. *Anaerobic dissimilation of glucose and xylose by Aerobacter indologenes*
Sugar fermented and products in millimoles per liter.

Substrate	Sugar fermented	Carbon dioxide	Hydrogen	Formic acid	Ethyl alcohol	Acetic acid	Lactic acid	Acetyl-methyl-carbinol	2,3-Butylene glycol	Succinic acid	Pctg. recovery carbon	Redox index
Glucose	123.8	189.0	34.2	34.6	82.5	1.1	3.68	0.89	79.4	0	97.0	.943
Xylose	99.73	113.3	19.0	26.2	55.8	11.27	5.16	0.91	44.0	5.43	98.5	.977

Medium = 2.0% sugar, 0.1% (NH₄)₂HPO₄, 0.5% NaHCO₃

TABLE 2. *Anaerobic dissimilation of glucose and xylose by Escherichia coli*
Sugar fermented and products in millimoles per liter.

Substrate	Sugar fermented	Carbon dioxide	Hydrogen	Formic acid	Ethyl alcohol	Acetic acid	Lactic acid	Succinic acid	Pctg. recovery carbon	Redox index
Glucose	73	41.2	39.2	4.25	27.0	22.05	87	3.56	95.5	0.97
Xylose	55.1	2.47	2.0	1.68	9.74	9.68	58.5	16.05	103	1.05

Medium = 2.0% glucose, 0.5% Bacto-peptone, 1.0% calcium carbonate.

In the work of Scheffer and Kluver, a scheme has been proposed to explain the mechanism of glucose dissemination by *Esch. coli*. Methylglyoxal, resulting from preliminary cleavage of the hexose molecule, stabilizes as lactic acid or is split into formic acid and acetaldehyde. The aldehyde serves as a precursor of ethyl alcohol and acetic acid. A direct 4- and 2-carbon splitting of the hexose molecule to give, intermediately, the dialdehyde of tartaric acid which subsequently undergoes rearrangement to succinic acid, is proposed to explain the formation of the 4-carbon acid. The proposed mechanism is based on the following assumptions:

1. If succinic acid is formed synthetically from intermediate 2-carbon compounds, each mole of succinic acid will be accompanied by two moles of 1-carbon compounds, i. e., formic acid or carbon dioxide. Then the total number of 1-carbon compounds will be equal in moles to the sum of (1) two times the succinic acid, (2) ethyl alcohol and (3) acetic acid.

2. If succinic acid is formed from a 4-carbon compound arising through a direct 4- and 2-carbon cleavage of the hexose molecule, then neither succinic acid nor the 2-carbon compound accompanying its formation will require simultaneous production of 1-carbon compounds. The sum of 1-carbon compounds will then be equal to the difference between the sum of 2-carbon compounds and succinic acid.

Results obtained by Scheffer on completed fermentations are in agreement with the latter assumption. Direct proof for such a mode of formation is lacking and absence of any evidence of the intermediary formation of such a compound as the dialdehyde of tartaric acid brings into question the proposed mechanism.

As with *Aerobacter*, it can be shown that the data in table 2 for the fermentation of glucose by *Esch. coli* comply satisfactorily with the Scheffer-Kluver scheme. The mechanism, however, is not adequate to explain all of the data obtained from the fermentation of xylose.

Qualitatively, the products obtained from the two sugars are the same. Quantitatively the most marked difference is in the much greater yield of succinic acid from xylose as compared with glucose.

The substrate-product ratio for the fermentation of xylose by *Esch. coli* (table 2) shows that lactic acid was formed at a rate only slightly greater than one mole for each mole of xylose fermented. The data are difficult to explain on any basis other than an initial cleavage of the pentose molecule into 3- and 2-carbon compounds, the 3-carbon fraction being converted into lactic acid. Other products, including succinic acid, would be the result of further conversions of the remaining 2-carbon fragment. The data favor the suggestion that *Esch. coli* can form succinic acid by synthesis from intermediate 2-carbon compounds.

DISCUSSION

Previously, this laboratory has reported the isolation of phosphoglyceric acid resulting from the action of members of the colon group on glucose, and conversion of the phosphoglyceric acid to pyruvic. (Werkman et al., 1936; Stone and Werkman, 1936). In view of these results it is reasonable to suppose that the chemistry of the primary reactions in the dissimilation of glucose by the coli-aerogenes group is similar to that proposed for muscle glucolysis by the Embden-Meyerhof scheme. Experimental data dealing with the primary reactions involved in the bacterial

decomposition of pentoses are lacking. It is, however, logical to assume that a preliminary phosphorylation precedes cleavage of the molecule to shorter carbon-chain fragments. Results reported here, particularly those obtained from the fermentation of xylose by *Esch. coli* indicate that the primary cleavage results in 3- and 2-carbon fragments. The results are best explained on the basis of the intermediate formation of pyruvic acid by way of phosphoglyceric acid, the pyruvic being subsequently reduced to lactic acid. The fermented xylose liquor gave a positive reaction for pyruvic acid when tested by the method of Simon and Piaux (1924), giving further support to pyruvic acid as an intermediate. The remaining products, including large quantities of succinic acid, must be formed through further conversions and synthesis from the remaining 2-carbon fraction.

In the fermentation of xylose by *A. indologenes*, the quantitative and qualitative similarity of the products with those derived from glucose indicates that primary cleavage results in similar intermediates in both cases. The previously reported results on the intermediary character of acetic acid in this fermentation, and results indicating that *A. indologenes* causes a hydrolytic cleavage of pyruvic acid to formic and acetic acids (Mickelson, Reynolds and Werkman, 1936) make it probable that a similar mechanism acts in the formation of acetylmethylcarbinol and 2,3-butylene glycol from xylose. In addition, the data indicate that succinic acid plays the rôle of an intermediate in the fermentation of xylose by *A. indologenes*.

CONCLUSIONS

The products formed in the fermentation of xylose and glucose by members of the colon-aerogenes group are, with one exception, qualitatively the same, but vary quantitatively. *A. indologenes* forms succinic acid from xylose, but not from glucose.

Data reported support an initial cleavage of the pentose molecule by *Esch. coli* and *A. indologenes* into 3- and 2-carbon fragments.

Esch. coli forms much larger quantities of succinic acid from xylose than from glucose.

It is probable that succinic acid formed from xylose by *Esch. coli* results through synthesis from intermediate 2-carbon precursors.

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EFFECT OF LONG CONTINUED TREATMENT ON THE ORGANIC MATTER, NITROGEN AND PHOSPHORUS CONTENT OF CLARION LOAM. I. CONTINUOUS CORN¹

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In spite of all the experiments which have shown the injurious effects of continuous cropping and the experiences of many farmers which have demonstrated the undesirability of the practice, there are still those who crop the land continuously to the same crop with no provision or at least with entirely inadequate provision for the return of the necessary fertility constituents to permit of the continued satisfactory use of the land for the growing of crops.

It has been found that the fertility of the soil can be maintained only if the organic matter content is kept up and some provision is made to return the plant food constituents removed by the crops grown. The cultivation and consequent aeration of the soil stimulates microbiological action and results in a rapid loss of organic matter and hence the alternation of non-cultivated with cultivated crops cuts down the rate of decomposition and tends to conserve the organic matter supply of the soil. There is also a greater return of crop residues with some crops than with others, and legume residues are especially valuable as sources of organic matter. It appears, therefore, that under a rotation of crops which includes a legume, fertility losses may be much reduced over those which occur under continuous cropping. It also appears that fertilization of the soil may lessen the rapidity of fertility losses and under continuous cropping the effects of soil treatments may be particularly significant.

The purpose of the work reported here was to study the losses of fertility under continuous cropping to corn with various soil treatments on plots at the Agronomy Farm of the Iowa Agricultural Experiment Station which have been under investigation since 1914. It was planned to determine the changes in organic matter, nitrogen, phosphorus, available phosphorus and pH in the soil on these plots from 1917 to 1936; to determine the effects of manure and limestone additions on these constituents; and also to determine the number of samples that should be taken from experimental plots one-tenth acre in size in order to insure significant and satisfactory results.

HISTORICAL

Blair and Prince (5) studied the effect of manure, nitrogen fertilizers and a mineral mixture consisting of superphosphate and muriate of potash on the supply of organic matter, nitrogen and phosphorus in soils. The amounts of organic matter and nitrogen in the soil were increased only on those plots which received manure in combination

¹ Journal paper No. J466 of the Iowa Agricultural Experiment Station. Project No. 447.

with the mineral mixture. Under all other treatments of the soil there was a loss of these constituents. In an earlier report of the same experiment (4, 8) it was noted that the nitrogen content of the soil was difficult to maintain under continuous cropping, even with additions of fertilizer.

Bear and Salter (3) concluded that the organic matter content of the soil could be maintained and increased by the use of fertilizers without plowing under green manure crops or crop residues other than the stubble left after the crops were harvested.

Bear (2) studied the effect of quicklime on the organic matter and nitrogen in the soil and noted that in every case the soils treated with quicklime and fertilizer contained a smaller amount of nitrogen and carbon than the soils receiving corresponding fertilizer treatments without the quicklime.

Salter (10) studied the influence of the carbon-nitrogen ratio of organic matter additions on the accumulation of the organic matter in the soil. The data indicated that additions of materials having a narrow carbon-nitrogen ratio resulted in a large accumulation of organic matter in the soil, whereas, materials having a wide ratio did not have anything like as great an effect.

Snider (13) studied the effect of long-continued soil treatment and crop rotation upon the nitrogen and organic matter contents of the soil. He reported that on lightly limed soils the total nitrogen and organic matter were maintained at a higher level than on untreated or highly limed soils.

White (18) found that where lime was used with manure or a complete mineral fertilizer, the temporary decrease in organic matter, resulting from stimulated bacterial action, was offset by the increased amount of crop residues.

White and Holben (19) studied the effect of caustic lime on soil treated with barnyard manure. They found that in 40 years soil treated with lime and manure produced 13,120 pounds of dry matter and 214 pounds of nitrogen more than soil treated with manure alone. They estimated that lime applied to manured soil had stimulated the decay of 2,000 pounds of organic matter beyond that occurring in soil treated with manure alone. This resulted in an increased yield of crops sufficient to bring about an accumulation of 4,245 pounds of crop residues, thus leaving a balance of 1,345 pounds of organic matter in excess of that in the soil receiving the manure treatment without lime.

Turk and Millar (16) studied the effect of various kinds and combinations of crop residues with and without lime and fertilizer additions on the organic matter and nitrogen content of some of the lighter soils of Michigan. They reported that greater losses of organic matter occurred with materials of low nitrogen content. This bears out the contention that it is necessary to add nitrogen to soil in order to increase the organic matter content to an appreciable extent.

Karraker (7) compared the effect of certain management practices on the amount of nitrogen in a soil. He found that soils left bare showed a loss of 530 pounds of nitrogen in the surface 18 inches over a period of 11 years; that with soils kept in continuous bluegrass, the nitrogen content remained about the same or showed a slight increase;

and that soils kept in continuous bluegrass and white clover showed a gain of 405 pounds of nitrogen.

Salter and Green (11) considered the effects of different crops and cropping systems on the soil and attempted to differentiate between the effects of cultural practices and the influence of the residues left by a crop. They assumed that the average effect of a given crop in a single year was proportional to the content of organic carbon and nitrogen in the soil at the beginning of the year. The losses of nitrogen and organic carbon from the soil under the different cropping systems arranged in decreasing order were continuous corn, continuous wheat, continuous oats, the 5-year rotation and the 3-year rotation. They estimated that about 10 per cent of the total nitrogen added in manure remained in a residual form in the soil and about 5 per cent of the organic carbon added remained undecomposed.

Metzger (9) showed that there was a close relationship between the total nitrogen content of the soil and crop production over a period of 25 years, and that there was a similar relationship between the organic carbon in the soil and crop production.

Snyder (14) observed that over a 4-year period there were greater losses of nitrogen under continuous wheat than under continuous corn, oats or barley. With clover in the rotation, and manure added, he was able to increase the nitrogen content of the soil. All soils under continuous crops lost humus, whereas, soils under a rotation and manure gained 0.2 to 0.5 per cent humus. He also found that the greatest losses of humus occurred in soils rich in nitrogen and humus and that the nitrogen content of soils becomes stabilized as the humus content decreases.

EXPERIMENTAL

The continuous corn experiment consists of five plots, each 28 feet wide, 155 feet long and separated by a 7-foot border. The plots were laid out on Clarion loam at the Agronomy Farm in 1914. The soil treatments made were as follows:

<u>Plot No.</u>	<u>Treatment</u>
906	Check.
907	8 tons manure once in 4 years.
908	8 tons manure once in 4 years plus sufficient limestone to neutralize the acidity of the soil.
909	Limestone sufficient to neutralize the acidity of the soil.
910	Check.

The manure was added at the rate of 8 tons per acre in 1914 and every fourth year thereafter. Limestone was added in 1914 and thereafter when necessary to neutralize the acidity of the soil.

Composite soil samples were taken from each plot in 1917. These samples were made by mixing 5 small samples taken from near the corners and center of each plot. In 1936 each plot was divided into

four sections 7 feet wide and 155 feet long. Three samples of soil were taken from each of the 4 sections of all plots. The samples were taken 50 feet apart, starting at random from 5 to 35 feet from one end of the plots. The soils were air-dried, ground and mixed thoroughly before any determinations were made. The pH, organic matter, total nitrogen, total phosphorus and soluble phosphorus were determined in duplicate on each sample. The results were calculated on a moisture-free basis.

The pH of the soils was determined electrometrically by the quinhydrone electrode. Total nitrogen was determined by the Gunning-Hibbard method (1). Inorganic carbon determined by the Schollenberger method (12) was subtracted from the total carbon determined by the wet combustion method (20) and the result multiplied by the factor 1.724 to give the organic matter. Total phosphorus was determined by the modified magnesium nitrate method (6), and the soluble phosphorus was determined by the 0.002 N sulfuric acid method (15).

RESULTS

1. *Loss of Organic Matter, Nitrogen and Phosphorus and Changes in Available Phosphorus and pH of Soils from 1917 to 1936.*

The results obtained in the determinations of organic matter, total nitrogen and phosphorus of the soil samples in 1917, the averages of those samples in 1936, and the changes in available phosphorus and pH are given in table 1.

TABLE 1. *Percentages of nitrogen, phosphorus and organic matter and pH changes and soluble phosphorus of soils samples in 1917 and 1936*

	Date	Check 906	Manure 907	Manure + lime 908	Lime 909	Check 910
Organic matter	1917	4.7959	4.2866	4.0169	2.8468	2.5011
Content	1936	3.8912	4.0187	3.0866	2.8815	2.1375
Difference		-0.9047	-0.1679	-0.9303	+0.0347	-0.3636
Nitrogen	1917	0.2221	0.1940	0.1901	0.1471	0.1322
Content	1936	0.1731	0.1701	0.1503	0.1327	0.1080
Difference		-0.0508	-0.0239	-0.0398	-0.0144	-0.0242
Total phosphorus	1917	0.0441	0.0413	0.0405	0.0335	0.0344
Content	1936	0.0354	0.0352	0.0346	0.0310	0.0284
Difference		-0.0087	-0.0061	-0.0059	-0.0025	-0.0060
Parts per million of available phosphorus	1917	35.00	35.00	24.50	25.00	24.10
	1936	22.55	23.88	33.75	19.76	Trace
Difference		-12.45	-11.12	+9.25	-5.24	-24.10
pH	1917	5.99	6.01	5.90	6.14	6.14
	1936	5.92	6.21	7.40	7.04	6.01
Difference		-0.07	+0.20	+1.50	+0.90	-0.13

The results obtained on the 1917 soils are from one composite sample, whereas, the figures for the 1936 results are the average of 12 samples. The results for the determinations on individual samples in 1936 are given in table 2.

TABLE 2. *Organic matter, nitrogen, phosphorus and pH of Soils Samples in 1936*

Plot No.	Sec-tion	Pctg. organic matter				Pctg. total nitrogen				Pctg. total phosphorus				p.p.m. soluble phosphorus				pH			
		Position of samples				Position of samples				Position of samples				Position of samples				Position of samples			
		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
906	A	3.6413	3.5920	3.8417		0.1528	0.1571	0.1651		0.0358	0.0349	0.0350		16.0	29.0	16.0		5.73	7.03	5.61	
	B	3.3759	4.3033	4.2148		0.1490	0.1702	0.1856		0.0335	0.0350	0.0363		16.3	28.1	28.9		5.78	6.34	5.63	
	C	3.5980	3.9339	4.1443		0.1714	0.1734	0.1770		0.0356	0.0384	0.0375		15.6	41.9	17.3		5.55	6.90	5.31	
	D	4.0231	3.9442	4.0835		0.1881	0.1827	0.1800		0.0352	0.0360	0.0319		15.6	31.7	14.2		5.44	6.29	5.54	
907	A	4.1636	4.2921	4.2420		0.1747	0.1689	0.1724		0.0339	0.0344	0.0321		20.8	26.0	15.6		5.71	6.03	5.82	
	B	4.0420	3.8965	3.9763		0.1694	0.1747	0.1661		0.0366	0.0333	0.0387		22.0	21.7	17.3		6.25	6.44	5.95	
	C	4.1887	3.8741	4.1156		0.1808	0.1691	0.1760		0.0349	0.0335	0.0355		23.1	23.1	18.9		6.75	6.39	6.27	
	D	4.0979	3.4635	3.8717		0.1735	0.1524	0.1637		0.0355	0.0353	0.0333		52.0	23.0	17.3		5.86	6.58	6.31	
908	A	3.4857	2.9739	2.8998		0.1577	0.1378	0.1631		0.0340	0.0321	0.0373		32.5	31.0	59.1		6.99	7.01	7.73	
	B	3.8377	1.9873	3.5265		0.1744	0.1130	0.1591		0.0379	0.0290	0.0371		44.6	27.1	28.9		7.62	7.57	7.35	
	C	2.8079	2.9419	3.3310		0.1735	0.1356	0.1592		0.0379	0.0317	0.0349		43.3	27.4	26.0		7.86	7.38	7.31	
	D	3.6146	2.3198	3.5132		0.1630	0.1095	0.1580		0.0375	0.0294	0.0359		35.1	27.7	22.2		7.51	7.55	6.97	
909	A	3.5606	2.1249	3.5196		0.1540	0.1067	0.1509		0.0331	0.0266	0.0348		20.8	23.6	20.8		6.59	7.27	7.05	
	B	3.7919	2.5665	2.9118		0.1625	0.1148	0.1380		0.0334	0.0279	0.0324		24.6	21.7	17.3		7.10	7.26	7.14	
	C	3.2957	1.6324	3.2108		0.1479	0.1030	0.1468		0.0322	0.0254	0.0361		20.4	17.3	20.8		7.23	7.39	6.95	
	D	3.0034	2.2022	2.7577		0.1424	0.1041	0.1216		0.0318	0.0269	0.0318		18.9	18.0	13.0		7.17	7.05	6.28	
910	A	2.3250	1.6861	2.9947		0.1033	0.0802	0.1350		0.0279	0.0237	0.0336		trace	trace	trace		6.10	6.01	5.82	
	B	2.2920	1.7372	2.5614		0.1589	0.0851	0.1233		0.0271	0.0244	0.0296		"	"	"		5.91	6.08	5.54	
	C	2.1731	2.5416	1.8663		0.1048	0.1174	0.0811		0.0287	0.0308	0.0265		"	"	"		5.94	5.69	6.26	
	D	2.4346	1.0955	2.1228		0.1146	0.0888	0.1034		0.0314	0.0278	0.0297		"	"	"		5.95	7.11	5.69	

The organic matter and nitrogen contents of the soils in 1917 were highest in the soil of plot 906 and lowest in that of plot 910, due mainly to the slope of the land, although there was some difference because of the variation in rate of decomposition of organic matter in the differently treated soils. The phosphorus content of these soils in 1917 was highest in the soil from plot 906 and lowest in the samples from plot 909, while in 1936 it was highest in the soil from plot 906 and lowest in that from plot 910.

In 1917 the available phosphorus of the soils was highest in the soils from plots 906 and 907, and lowest in that from plot 910. In 1936 the available phosphorus content was highest in the soil from plot 908, and only a trace was found in the soil from plot 910.

The pH of the soil from all plots was about the same in 1917, ranging from pH 5.90 to pH 6.14. But in 1936 the pH of the soils which had received lime (plots 908 and 909) was increased considerably over that of the soils which had not been treated with lime. The soil treated with manure was slightly less acid and the two check soils were slightly more acid in 1936 than in 1917.

2. Crop Yields.

The yields of corn calculated on a 15 per cent moisture content from 1915 to 1936, inclusive, are given in table 3.

TABLE 3. *Corn yields, bushels per acre, continuous corn, 1915-1936*

Year	No. 906	No. 907	No. 908	No. 909	No. 910
	Check	Manure	Manure + lime	Lime	Check
1915	38.6	36.8	41.2	19.2	10.5
1916	36.0	35.6	39.6	30.8	14.0
1917	48.7	50.6	51.8	47.2	40.0
1918	25.7	37.1	38.6	31.4	24.3
1919	38.6	55.7	60.0	42.8	37.1
1920	41.4	50.0	64.2	50.0	45.7
1921	34.2	48.5	50.0	42.9	35.7
1922	38.6	48.5	52.8	45.7	32.8
1923	30.0	51.3	52.5	43.8	36.3
1924	28.5	35.7	34.2	30.0	22.8
1925	50.6	53.3	57.3	45.3	25.3
1926	32.5	32.5	35.0	28.8	17.5
1927	33.3	34.7	37.3	32.0	25.9
1928	47.6	48.5	44.8	35.0	28.7
1929	39.6	44.7	45.2	36.3	30.4
1930	25.9	27.0	30.9	29.0	28.1
1931	35.8	45.5	64.7	35.1	29.7
1932	44.5	50.0	48.8	40.6	31.7
1933	44.3	49.3	47.5	35.6	26.9
1934	16.8	17.4	15.8	17.0	14.0
1935	52.6	59.1	62.7	60.4	40.4
1936	18.3	19.3	22.3	20.9	15.0
Average	36.4	42.3	45.3	36.4	27.4

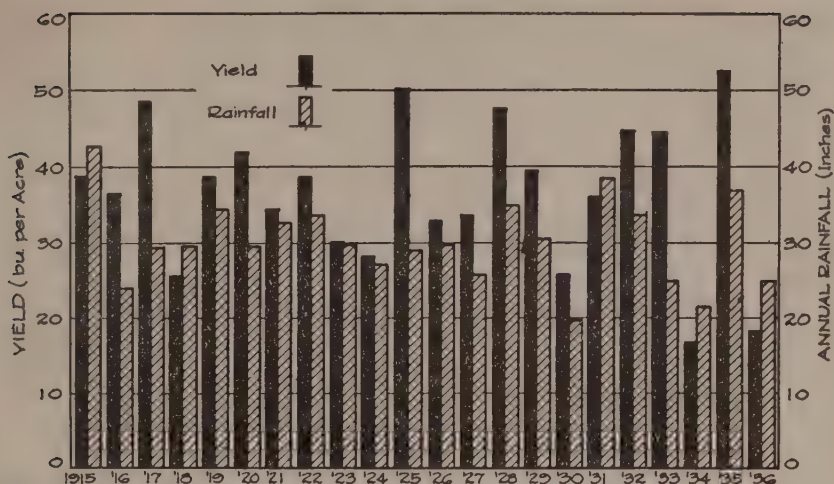


Figure 1. Relation of yield to rainfall. (Plot 906 for 1915-1936).

The yields on all plots varied considerably from year to year mainly because of weather conditions, but the relative yields on the variously treated plots were much the same in any one year.

The annual rainfall for the period from 1915 to 1936, inclusive, and the relation of yield to rainfall are shown for plot 906 in fig. 1.

The data in the graph show a fairly close relation between the average annual rainfall and the corn yield, but this was not always true. For example, the total rainfall in 1918 was practically the same as in 1917, but the yield of plot 908 was considerably reduced. Undoubtedly this was due to the distribution of the rainfall in 1918. The average yields of the different plots show the same trend of yield for the plots as was shown by the individual crops.

3. Sampling Technique.

One of the objectives of this work was to determine the number of samples which would be required for a test of significance between the differences in organic matter, nitrogen, phosphorus and pH at the different samplings.

There are two methods of procedure sometimes employed in uniformity trials which may be adapted for this purpose. The first method consists in taking a number of samples for analysis from each plot and determining the coefficient of variability (standard deviation divided by the mean difference). From the relationship of the coefficient of variability to probability the number of observations necessary for a test of significance can be calculated. The other method consists of making frequency distributions of the averages of the combinations of 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 observations made on one of the constituents on the 12 samples from a plot that showed a large variation, and then selecting the smallest number, the average of combinations of which comes sufficiently close to being the same as the average of the 12 individual

samples. Both methods are based on certain assumptions. In the first method the magnitude of the difference for significance must be assumed or an infinite number of samples may be necessary to prove significance and in the second method it is assumed that the number of observations made are in excess of that required for an adequate test of variability.

For the estimation of the number of samples that should be taken to show significance in the difference between variates by the first method, values were calculated for organic matter, nitrogen, phosphorus and pH for each of the five plots and the results obtained are presented in table 4.

To illustrate the second method of determining the number of observations necessary to sample the soil adequately, combinations of 2, 3, 9 and 10 samples were made on the results obtained for organic matter in plot 909. The distribution of the averages of these combinations is shown in figs. 2, 3 and 4.

DISCUSSION OF RESULTS

Since there was only one sample taken from each plot in 1917, the data do not permit of a test of the significance of the differences between the 1917 and 1936 determinations. However, the differences between the various soils are fairly consistent for both samplings and it is believed that they are large enough to be of some practical value even though a test of significance cannot be made.

If it is assumed that the variation of the soil in organic matter, nitrogen and phosphorus in 1917 was the same as that found in 1936, the significance of the losses of these constituents can be tested. A test of

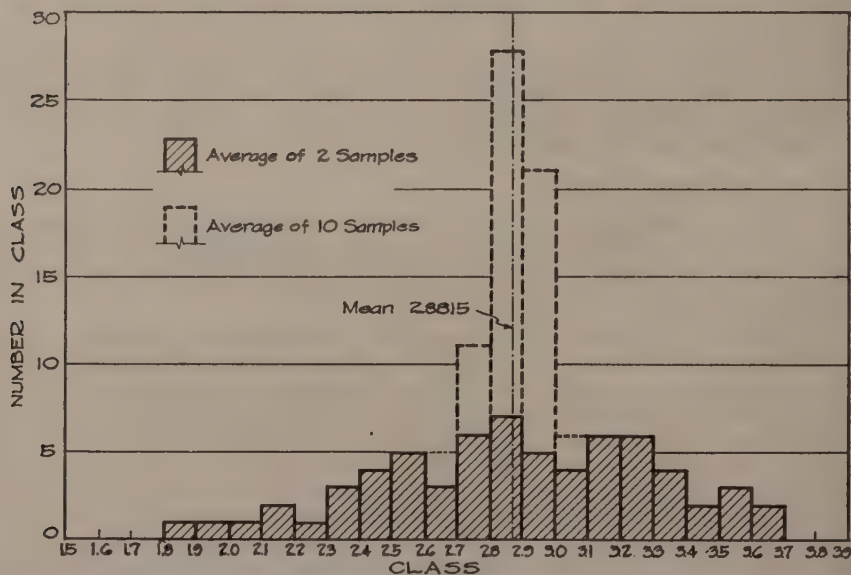


Figure 2. Distribution of averages of combinations of 2 and 10 samples per plot for organic matter of soil in plot 909.

TABLE 4. Coefficient of variability and the number of samples necessary to show significance

	Plot No.									
	906		907		908		909		910	
	s/mD*	No. samples 5% pt. 1% pt.	s/mD	No. samples 5% pt. 1% pt.	s/mD	No. samples 5% pt. 1% pt.	s/mD	No. samples 5% pt. 1% pt.	s/mD	No. samples 5% pt. 1% pt.
Organic matter	0.308	4 5	1.33	16 26	0.606	6 9	18.8		1.40	18 30
Nitrogen	0.25	3 5	0.304	4 5	0.54	5 8	1.5	20 34	0.97	10 16
Phosphorus	0.19	3 4	0.32	4 5	0.54	5 8	1.38	18 30	0.47	5 7
pH	8.2		1.69	26 44	0.19	3 4	0.347	4 6	2.5	52 87

* Coefficient of variability.

this kind was made based on the above assumption and it was found that the losses in organic matter between 1917 and 1936 were highly significant in the soils of plots 906 and 908, that significant losses in nitrogen and phosphorus occurred in all soils, except that of plot 909, that significant differences in the soluble phosphorus existed in all soils, except that of plot 909, and that there was no significant differences in the pH of the soils in 1936 from that in 1917, except in the soils treated with lime (plots 908 and 909). Where the mean difference was fairly large and the standard deviation was small, the differences were significant or highly significant. In several cases where the differences were found to be highly significant they would have been significant even with a larger standard deviation than that found in 1936. This would permit of a larger variation in the soils sampled in 1917 than probably existed. Even though this test is based on certain assumptions there are indications that the differences are significant.

The soil in plot 906 showed a large loss of organic matter, nitrogen and phosphorus, a considerable decrease in available phosphorus, and a slight decrease in pH. A relatively large loss of organic matter would be expected over a 19-year period since the organic matter content was relatively high in 1917, and no addition of organic material was made, except in the crop residues. The pH of this soil was 5.99 in 1917 and 5.92 in 1936, and it is high enough to allow considerable decomposition, yet there is not as much as would be expected at a higher pH. The loss of nitrogen was also relatively large and correlated with the loss of organic matter. The loss of phosphorus from the soil in this plot (906) was quite large, but it cannot be assumed that the phosphorus

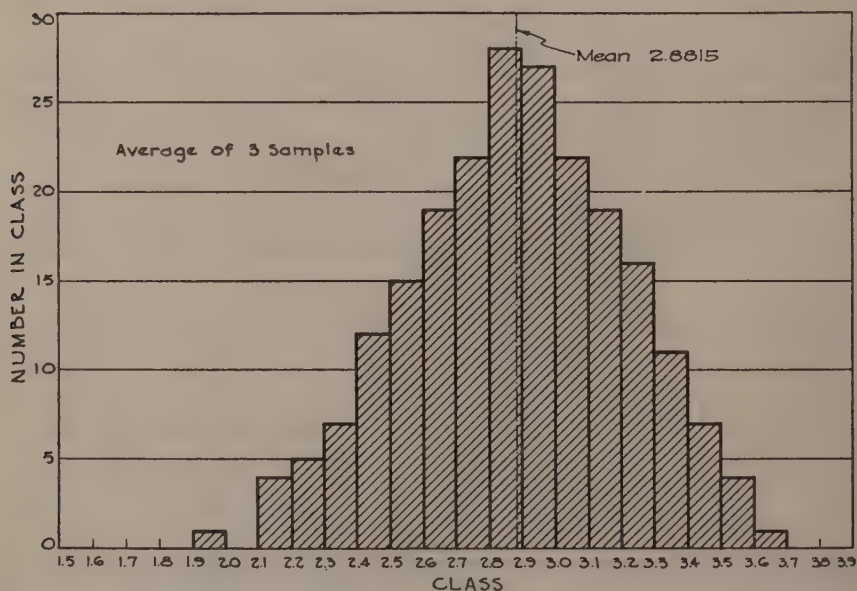


Figure 3. Distribution of averages of combinations of three samples per plot for organic matter of soil in plot 909.

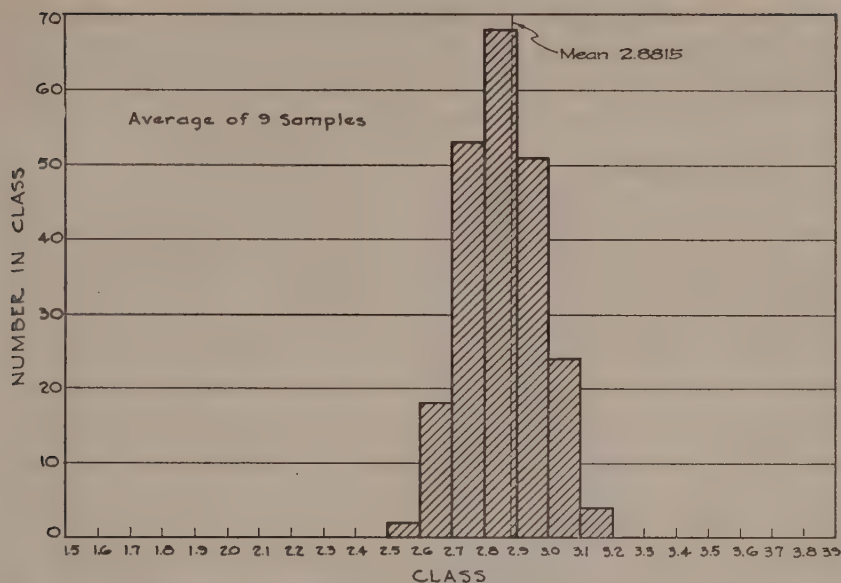


Figure 4. Distribution of averages of combinations of nine samples per plot for organic matter of soil in plot 909.

removed in the crop harvested during the 19-year period was all removed from surface soil. There was a large decrease in the amount of available phosphorus in this soil, which was largely independent of the amount of total phosphorus present. The pH of the soil was slightly lower in 1936 than in 1917, but the change was probably insignificant.

There was a loss of both organic matter and nitrogen in the soil of plot 907 from 1917 to 1936, but this loss was not as great as in the soil of plot 906. The net loss of organic matter should be small in this soil over a period of years because of the large amount added in manure and crop residues. The microbiological activity in the soil would be increased because of the large number of microorganisms introduced with the manure and the large amount of readily available organic matter. However, the pH of the soil was 6.01 in 1917 and 6.21 in 1936 and the microbiological activity would not be as great as in a neutral soil. Likewise, the net loss of nitrogen would be small because of the relatively large addition of nitrogen in the manure. The loss of nitrogen was also correlated fairly closely with the loss of organic matter in this soil. The loss of phosphorus in this soil was not large considering the total crops harvested from the plot, and also because there was some addition of this constituent in the manure. There was a rather large decrease in available phosphorus in this soil, but the decrease was not as large as in the check soil, plot 906.

The soil of plot 908 showed a relatively large loss of organic matter during the period from 1917 to 1936. Since this plot received larger crop residues than any of the other plots, and a total amount of 48 tons of manure per acre during the time the experiment has been in opera-

tion, it was not expected that the net loss from this soil would be so great. However, the pH of the soil was 7.4, and this high pH, together with the large number of microorganisms introduced into the soil with the manure undoubtedly account for the large decomposition of organic matter in the soil of this plot. The loss of nitrogen in this soil was quite large as would be expected since the loss of organic matter was also relatively large. This loss of nitrogen would have been larger, no doubt, if none had been added in the manure. Also, the increased bacterial numbers probably caused more nitrogen to be fixed or held in the soil than would have been the case with a less active microflora. The net loss of phosphorus from the soil in plot 908 was no larger than would be expected from the amount present in the crops removed and that added in the manure. The available phosphorus was increased considerably in the soil of this plot. This increase in availability of phosphorus may be attributed to the combined effect of manure and lime.

In the soil of plot 909 there was an apparent tendency toward the conservation of the constituents determined. The analyses showed a slight increase in the organic matter content in 1936 over that in 1917, but the amount was small and hardly significant. Since this soil was treated with lime, it would be assumed that there should have been a considerable loss of organic matter on account of the higher pH and increased bacterial action. However, the content of organic matter was so low in the beginning that the decomposition would not be rapid. The inference drawn is that while the lime stimulated decomposition, this was offset by the fact that the organic matter present was apparently in an advanced stage of decomposition and hence the rate of decomposition was very low. The loss of nitrogen from the soil in this plot was fairly small as would be expected since there was only a slight change in the organic matter content. The increased pH may have stimulated nitrogen fixation in this soil and as a result of this increased fixation the net loss of nitrogen was small. The loss of phosphorus in this soil for the 19-year period was small and probably less than that removed in the crops harvested. This small loss of total phosphorus suggests another interpretation of the small loss of organic matter and nitrogen from the soil of this plot. Van Slyke (17) calculated that about 4.0 pounds of phosphorus was removed by the grain in a 25-bushel crop of corn. The total yield of corn on this plot was 722.6 bushels for the 19-year period and would contain then 115.6 pounds of phosphorus. The analysis showed a loss of only 50 pounds. Here again the analyses represent the phosphorus in the surface soil and the phosphorus removed in the crop was probably not all removed from this layer. However, the data suggest the sample taken in 1917 was not representative of the soil of this plot. The same conclusion, namely, that the samples were not representative, may be drawn from the analyses for organic matter and nitrogen. In other words, the amounts of organic matter, nitrogen and phosphorus were probably higher in 1917 than appears from the analyses of the samples taken this year and a greater loss should have been shown as a result of the treatment with lime.

The loss of organic matter and nitrogen from the soil of plot 910 was quite large, yet not as large as expected. The organic matter in this

soil also may have been in an advanced stage of decomposition and the rate of decomposition very slow. The available phosphorus was greatly reduced in this plot during the 19-year period, only a trace being present in the soil samples in 1936. The loss of total phosphorus in this soil was little more than would be expected by the crops removed.

Because of the ordered arrangement of the plots in the field, no test of significance of the differences brought about by treatments can be made. However, the effects of the manure and lime on the organic matter, nitrogen, total and available phosphorus and on the pH are quite obvious, especially in the case of plots 907 and 908.

The manure added to plot 907 tended to conserve the organic matter, nitrogen and total phosphorus content of this soil. There was more organic matter decomposed in the soil of plot 907 than that in plot 906, but this was offset by the manure added and the larger amount of crop residues returned. The manure additions tended to conserve the nitrogen and the net loss of nitrogen in this soil was less than half that observed in the soil of plot 906. The smaller loss of phosphorus in this soil than in the adjoining check soil was undoubtedly caused by the addition of this constituent in the manure. The pH of the soil treated with manure was increased 0.2 over that of the adjoining check soil.

The effects of manure when added with lime, as shown by the analyses of the soil in plot 908, were quite different than when the manure was added alone. The loss of organic matter was large in this plot in spite of the application of a total of 48 tons of manure per acre and the return of relatively large amounts of crop residues. The loss of nitrogen was larger in this soil than in that treated with manure alone and parallels the decrease in the organic matter content. The effect of manure alone or lime alone was to decrease the availability of phosphorus, but with manure and lime together the effect was to increase the available phosphorus content considerably. The pH of the soil was increased more where both manure and lime were added than where either material was added alone.

The losses of organic matter, nitrogen and phosphorus in the soil of plot 910 were similar to those obtained in the soil of plot 906, but were not quite as large, since the total amounts of these constituents in this soil were much lower than in the soil of plot 906. The organic matter was probably present in an advanced stage of decomposition and the rate of loss less than in the soil of plot 906. The acidity of this soil, the low content of total phosphorus and organic matter were no doubt contributing factors in the decrease in available phosphorus.

In the beginning of the experiment higher yields were obtained on plots 906, 907 and 908 than on plots 909 and 910. This corresponds to the original fertility of the plots. At the end of 22 years the average yield of plot 907 was greater than that for plot 906 or plot 909.

The highest yield was obtained on plot 908 in the beginning of the experiment, and except for slight variations the yield on this plot was highest each year, the average yield for the 22-year period being higher than the yield on any other plot.

The yield on plot 910 was lower in the beginning of the experiment than that on any other plot and continued to be low throughout the ex-

periment. During the last ten years, even though the yield was sometimes fairly high, the quality of corn was very poor.

The results of the determination of the number of samples required for significance by the first method mentioned require some explanation. The purpose of this work was not to determine the number of samples necessary to prove significant mean differences alone, but to estimate the number of samples needed to test significance where the mean difference was large enough to give a significant test with 12 samples. In the soil of plot 909 the change in the organic matter content was only 0.0337 per cent, and it is obvious that an infinite number of samples would be needed here to give a significant test. With a large standard deviation and a small mean difference, the number of samples will be large.

The loss of organic matter was large in the soil of plots 906 and 908. With the standard deviation found in 906, only 5 samples were necessary to give a proof of significance, whereas, in plot 908, 9 samples were necessary. The mean difference in organic matter content in the soil of plot 908 in 1917 and 1936 was greater than in plot 906, but the standard deviation was larger in plot 908 than in plot 906, hence a larger coefficient of variability was obtained and therefore a larger number of samples was necessary.

The number of samples for organic matter, nitrogen, phosphorus and pH necessary to give a highly significant test, where the mean difference was large, ranged from 4 to 9, and for significance the number ranged from 3 to 6. In the cases where the mean difference was small and where the standard deviation was large the number ranged from 16 to an infinite number.

These results indicate that 9 samples would be sufficient for all the plots to provide representative samplings and to give a test of significance where the changes were large. For the plots showing a small change it is quite obvious that those differences are not significant and it is useless to take a large number of samples just to prove them significant. This number (9 samples) was selected because it appeared in a plot with a large mean difference, yet where the variation was great. Also it is assumed that the standard deviation for other plots to be samples will be about the same or less than for the organic matter of plot 909, and that the mean differences will be rather large. This method does not show the exact number of samples that should be taken, but it is a means of making an estimation by observing the variation in sampling.

The second method for estimating the number of samples for an adequate test of the variability of the soil in these plots does not give the exact number but it serves as a basis for obtaining a fair estimate of the number required. The results of the determinations of the organic matter content of the soil of plot 909 were used to illustrate the principle of this method because the variability of the soil in this plot was large. If the number of samples needed for an adequate test is determined for a soil of large variability the number found will be large enough for all plots with an equal or smaller amount of variation.

The range of the content of organic matter for the soil of plot 909 for the 12 samples was from 1.63 per cent to 3.80 per cent and the mean was 2.8815 per cent. Sixty-six combinations of two samples each can be made from these 12 samples. By taking the averages of these combinations, the

range was found to vary from 1.88 per cent to 3.68 per cent and the averages were well scattered in this range. Only seven of these combinations had averages which were between 2.8 and 2.9 per cent. Therefore, the chance of drawing two samples with an average in this range is very small. The average of two samples is very likely to be as low as 2.3 or as high as 3.5 and there is some chance that it may be outside this range. Therefore, results based on two samples from this plot would not be reliable since they do not give a sufficient test of the variation within the plots.

Two hundred twenty combinations of 3 samples each can be made from 12 samples. The averages of combinations of 3 samples ranged from 1.9 per cent to 3.7 per cent. Only 28 of these combinations had averages which were between 2.8 and 2.9 per cent and only 72 of the 220 were between 2.7 and 3.0 per cent. The chance of drawing 3 samples with an average in even the latter range is small.

Two hundred twenty combinations of 9 samples each can be made from 12 samples. The averages of combinations of 9 samples ranged from 2.5 per cent to 3.2 per cent. Sixty-eight of these combinations had averages which were between 2.7 and 2.8 per cent and 172 had averages between 2.7 and 3.0 per cent. In other words, approximately 8 out of 10 combinations of 9 samples had averages between 2.7 and 3.0 per cent.

Sixty-six different combinations of 10 samples each were made from the 12 samples taken and each of these was averaged. The lowest average was 2.7 per cent and the highest was 3.1 per cent. The tendency was for the average of 10 samples to be near the mean (2.8 per cent) of the 12 samples as would be expected. The chance of taking 10 samples from this plot and getting about the same information as was found with 12 samples is fairly high, and the information added by the other 2 samples is probably not enough to make the extra sampling worth while.

SUMMARY AND CONCLUSIONS

A composite sample of soil was taken from each plot of the continuous corn experiment at the Agronomy Farm of the Iowa Agricultural Experiment Station in 1917. The composites were made by mixing thoroughly 5 small samples taken from near each corner and the center of each plot. In 1936 a different sampling technique was employed. The method followed consisted in dividing each plot into 4 parts and taking 3 samples 50 feet apart in each of these sections, starting at random from 5 to 35 feet from one end of the plots. The 12 samples taken from each plot were analyzed separately to determine the variability of the soil in the plots.

The samples taken in 1917 and those taken in 1936 were analyzed for organic matter, nitrogen, total phosphorus, available phosphorus and pH. Although the data do not permit of a test of the significance of the differences between the content of these constituents found in the soils sampled in 1917 and those sampled in 1936 these differences appear to be of considerable practical importance. The results obtained may be summarized briefly as follows:

1. The organic matter, nitrogen and phosphorus in the soils in all plots decreased from 1917 to 1936, except in the soil which received a treatment of lime alone, where the organic matter was increased slightly.

2. The largest decrease in organic matter occurred in the soil which had received applications of manure and lime. In all but one case the loss in organic matter was greatest in those soils which contained the greatest amount of this constituent at the beginning of the experiment. Only in the soil which received lime alone was it found that this did not hold true.

3. The addition of manure to the acid soil reduced the loss of organic matter and nitrogen.

4. The addition of manure and lime to the acid soil decreased the loss of nitrogen.

5. The addition of manure alone or lime alone decreased the amount of available phosphorus in the soil, whereas the addition of both manure and lime increased the amount present.

6. The results indicate that lime tended to conserve the organic matter, nitrogen and phosphorus of the soil which received lime alone, although the results were not conclusive.

7. Considerable variability in the soil within the plots was found. Two methods were employed to estimate the number of samples necessary to sample adequately the soil of all plots and to give a test of significance of the differences.

8. It was estimated by these methods that 9 samples from each plot would be necessary to sample adequately the soil of plots which showed as much variation as was found in these continuous corn plots.

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SOME TINGITIDAE (HEMIPTERA) FROM OCEANIA

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The present paper is based largely upon specimens of Tingitidae kindly sent to the writers by the late C. F. Baker of the Philippine Islands and Dr. L. B. Uichanco, Head of the Department of Entomology, University of the Philippines, as well as some other miscellaneous material from Oceania kindly sent by various collectors. These collections contain more than 250 specimens, representing 16 genera and 35 species. Of the 35 species, 7 are described below as new. The holotypes and allotypes are in the Drake collection.

Phatnoma pacifica Kirkaldy

Phatnoma pacifica Kirkaldy, Proc. Linn. Soc., N. S. W., Sidney, XXXIII, 1908, p. 363.

Phatnoma pacifica Hacker, Mem. Queens. Mus., IX, 1928, p. 176, Pl. XX, fig. 4.

One male, Los Banos, Luzon, Philippine Islands, Dec. 1, 1926, collected by E. B. Jamora. This specimen agrees with Hacker's figure of the species.

Serenthia sedalis Drake

Serenthia sedalis Drake, Philip. Jour. Sci. XXXIV, 1927, p. 312.

One specimen, Mt. Makiling, Luzon, Philippine Islands, collected April 14, 1931, at an elevation of 150 meters by L. B. Uichanco.

Monanthia sauteri Drake

Monanthia sauteri Drake, Ohio Jour. Sci., XXIII, 1923, p. 103.

Los Banos, Luzon, Philippine Islands, 1 specimen, May 10, 1915, collected by C. S. Banks; 7 specimens, June 28, 1922 and January 15, 1929, collected on *Cordia myxa* L. (Boraginaceae) by L. B. Uichanco, and one example April 22, 1930 by V. J. Madrid.

Monanthia evidens Drake

Monanthia evidens Drake, Philip. Jour. Sci., XXXII, 1927, p. 53.

Twelve specimens from Los Banos, Luzon, Philippines, elevation 50 meters, collected on *Ehretia acuminata* R. Br., May 15, 1934, by L. B. Uichanco. This species was known heretofore only from the holotype (female) from Imugan, Neuva Vizcaya, P. I. The male (allotype) is very similar to the female in general appearance, color and structure and differs only in being of slightly smaller size.

***Monanthia seorsa*, sp. nov.**

Ovate, greyish brown, with fuscous markings. Head black, the spines short, curved, testaceous, the median tubercular. Body beneath black. Rostral channel moderately wide, rather deep, open behind, the laminae yellowish brown. Rostrum long, brown, dark at apex, extending to end of sulcus. Antennae rather short, moderately slender; segment I short, moderately stout, black-ferruginous; II shorter and more slender, dark reddish brown; III slender, testaceous, two and one-half times as long as four; IV dark fuscous, strongly swollen towards tip.

Pronotum moderately convex, concealed by the closely overlapping paranota, except in front and behind; triangular projection fuscous; lateral carinae as exposed behind testaceous, divaricating; median carina sharply raised, brownish behind; triangular exposed portion in front slightly elevated, the anterior margin subtruncate; paranota very broad, resting closely upon dorsal surface of pronotum, the outer edges meeting on disc and there concealing median carina, thence posteriorly with margin raised moderately. Elytra extending a little beyond apex of abdomen; costal area moderately wide, irregularly biseriate, the transverse nervures thickened, placed in groups of two to three and these groups connected with stout, transverse nervelets; subcostal area broad; discoidal area large, extending very deeply, broadly and concavely into the subcostal area at the apex, the anterior and hind margins of the concave projection strongly raised and dark fuscous. Legs rather short; femora mostly black, very granular, the tibiae and tarsi testaceous, the tips of the latter dark.

Length, 3.00 mm.; width, 1.44 mm.

Holotype (male) and allotype (female) Taiwan (Formosa). The paranota and costal area separate this species at once from its nearest allies.

***Monanthia seorsa inflata*, n. var.**

Differs from typical *seorsa* in having humeri more prominent, the outer portion of paranota behind the disc much more strongly raised and inflated, and the anterior and posterior margins of the concave projection of discoidal area considerably more raised than in the female of *seorsa*. Other structures and color like *seorsa*.

Length, 3.30 mm.; width, 1.68 mm.

Holotype (female) Los Banos, Luzon, Philippine Islands, April 21, 1930, collected by F. Villaneuva. Two paratypes, one female taken with type, and the other female taken from same locality, April 22, 1930, by V. J. Madrid.

***Monanthia sessoris*, sp. nov.**

Uniformly greyish testaceous, sparsely clothed with very fine, short, pale, recumbent hairs. Rostral laminae testaceous, slightly divaricated posteriorly, widely separated behind; rostrum brownish, black at tip, extending a little beyond apex of channel. Body beneath brown, rather densely clothed with short pale hairs. Head dark, with five slender, testaceous, suberect spines. Antennae with first two segments short, brownish, the others wanting. Legs slender, yellowish brown, the tips of tarsi dark.

Pronotum moderately convex; median carina strongly raised, composed of one row of rather large, rectangular areolae; hood small, faintly produced forward at middle; paranota broad, resting tightly upon dorsal surface of pronotum, the outer margin practically touching median carina; lateral carinae as exposed behind nearly parallel. Costal area broad, biseriate (a few extra cells on one side), the areolae large and hyaline; subcostal area narrower, biseriate; discoidal area extending considerably beyond middle of elytra, bounded by a raised nervure, narrowed both at base and apex, broadest near middle, there five areolae deep. Wings slightly clouded, iridescent, a little longer than abdomen.

Length, 2.85 mm.; width, 1.20 mm.

Holotype, female, Mt. Makiling, Luzon, Philippine Islands. The strongly elevated median carina and fine hairs on nervures are very distinguishing characters.

***Monanthia uichancoi*, sp. nov.**

Color and form very similar to *M. evidens* Drake but differs from it in being slightly smaller and especially in the character of paranota, presence of lateral carinae, and the much more elevated hind portion of median carina. Paranota with transverse nervure above humeri and inner nervure along outer margin of cells black and very sharply raised so as to form carina-like ridges, the areas within the angles formed by the ridges, both in front and behind, moderately impressed. Lateral carinae exposed on triangular process, distinct but not strongly raised, pale in color and divaricating posteriorly. Median carina strongly raised on triangular process, there uniseriate. Hood small, more sharply raised than in *evidens* Drake.

Elytra narrower than in *evidens*; discoidal area very similar in form to *evidens*. Antennae slender, pale testaceous, basal segment short, brownish black, slightly stouter and shorter than II; III tending to be a little longer in male than in female.

Holotype (male), allotype (female) and nine paratypes, Calamba, Luzon, Philippine Islands, at an elevation of 25 meters, Nov. 10, 1929, collected by L. B. Uichanco. This species is named in honor of Mr. L. B. Uichanco who has taken a very active and unusually keen interest in the insects of the Philippine Islands.

***Physatochila marginata* (Distant)**

Teleonemia marginata Distant, Ann. Soc. Ent. Belg., LIII, 1909, p. 121.

Cysteochila (*Parada*) *marginata* Horvath, Ark. Zool., XVII, 1925, p. 3.

Physatochila marginata Bergroth, Rev. Russ. Ent., XVII, 1917, p. 105.

Baguio Benguet, Philippine Islands, 2 examples, C. F. Baker. This is not a very typical species of the genus *Physatochila*.

***Cysteochila bakeri* Drake and Poor**

Cysteochila bakeri Drake and Poor, Philip. Jour. Sci., LXII, 1937, p. 9.

Mt. Makiling, Luzon, female, C. F. Baker. This specimen bears the same data as the male.

Cysteochila abundantis Drake and Poor

Cysteochila abundantis Drake and Poor, Philip. Jour. Sci., LXII, 1937, p. 8.

Mt. Makiling, Luzon; Tanglecolan, Bukidnon; and 2 examples from Tigao, Mindanao, Philippine Islands, C. F. Baker; Nilchiri, Coondor, 1902, 1 specimen, collected by M. Maindron. One specimen, Pasonanca, Zamboanga, Philippine Islands, April 9, 1936, by L. B. Uichanco. This is one of the most common members of the genus in the Philippines.

Cysteochila elongata Distant

Cysteochila elongata Distant, Ann. Soc. Ent. Belg., XLVII, 1903, p. 49.

Takao, Taiwan (Formosa), 1 male. The writers propose the name *Baeochila* subgen. nov. for *elongata*. This subgenus has a rather large hood which distinguishes it from typical *Cysteochila*. The metasternal canal is distinct. The rostral sulcus is open behind.

Cysteochila javensis, sp. nov.

Moderately large, elongate-ovate, brownish, with greyish white markings. Pronotum convex above, coarsely pitted; tricarinate, each carina uniseriate; median carina slightly more elevated, the areolae moderately large; lateral carinae faintly converging behind, concealed by paranota in front; paranota moderately large, tumid, embrowned above; hood small, faintly produced forward in front.

Head with five short, blunt, testaceous spines. Segments I and II of antennae short, pale brown, the first slightly longer and stouter; III long, slender, testaceous; IV wanting. Bucculae yellowish brown, closed in front. Rostrum extending to metasternum, brownish, black at apex; rostral laminae testaceous, the channel wide and chordate on metasternum. Elytra brownish, the basal portion and a small area at apex of discoidal area greyish white; costal area narrow, uniseriate, the transverse nervelets infuscate, subcostal area biseriate; sutural area impressed, widest a little behind middle, there four areolae deep; sutural area more widely reticulate behind. The principal nervures and sides of paranota and hood sparsely clothed with pale hairs.

Length, 2.80 mm.; width, 1.00 mm.

Holotype (female), and 1 paratype, Pekalongan, Java, 1907, F. Muir.

This species differs from *C. bakeri* D. & P. in having a smaller hood and paranota, narrower costal area and duller color; from *C. lecta* D. & P. by its narrower costal area and more inflated and more elevated paranota.

Perissonemia recentis Drake and Poor

Perissonemia recentis Drake and Poor, Philip. Jour. Sci., LXII, 1937, p. 5.

Singapore, Straits Settlements, female, collected by C. F. Baker. This specimen was undoubtedly collected with the type and bears the same data.

Perissonemia torquata Drake and Poor

Perissonemia torquata Drake and Poor, Philip. Jour. Sci., LXII, 1937, p. 2.

Butuan Mindanao, Philippine Islands, female, taken by C. F. Baker. This specimen agrees with the holotype.

Perissonemia borneensis (Distant)

Teleonemia borneensis Distant, Rec. Ind. Mus., III, 1909, p. 166, Pl. X, figs. 1, 1a.

Perissonemia borneensis Drake & Poor, Philip. Jour. Sci., LXII, 1937, p. 4.

Sandakan, Borneo, female; Singapore, Straits Settlements, male and female, C. F. Baker; Zamboanga, Mindanao, Philippine Islands, male, C. F. Baker; Island Sibuyan, male, C. F. Baker; Malaba, male, 1902, taken by M. Maindron.

Perissonemia illustrus Drake and Poor

Perissonemia illustrus Drake & Poor, Philip. Jour. Sci. LXII, 1937, p. 4.

Imugan, N. Vizcaya, Philippine Islands, 3 examples, C. F. Baker. These specimens were collected with the type series.

Perissonemia vegata, sp. nov.

Small, brownish black, the pronotum in front and a conspicuous spot on each side at the base of triangular process covered with whitish exudation. Head very dark brown, the spines much reduced. Eyes dark. Rostrum dark brown, black at apex, extending between intermediate legs. Body beneath dark brown. Legs dark brown, the tarsi lighter. Antennae moderately long; segments I and II dark ferruginous, the first segment twice as long as and considerably stouter than the second; III testaceous, twice as long as IV, the latter black.

Pronotum black, strongly convex above, coarsely pitted, the paranaota wanting; collar raised, reticulate, truncate in front; median carina sharply raised, non-reticulate; lateral carinae indistinct, perhaps faintly discernible behind; triangular process reticulate, truncate at apex. Elytra constricted beyond middle, brownish black, at the base and constriction brownish; costal area extremely narrow, very indistinctly areolate; subcostal area triseriate; discoidal area widest beyond middle, there five aerolae deep; sutural area much more widely reticulated, the areolae considerably clouded with fuscous. Wings longer than abdomen, clouded with fuscous.

Length, 2.85 mm.; width, 1.00 mm.

Holotype, female, Sandakan, Borneo, C. F. Baker. This species belongs to the subgenus *Ulonemia* Drake and Poor. It may be separated at once from *borneensis* (Distant) by the extremely narrow costal area and the much darker and more tumid pronotum.

Perissonemia tasmaniae, sp. nov.

Allied to *P. recentis* D. & P. but much more slender, pronotum not so strongly convex, carinae slightly more elevated and thicker. Brown, costal area and pronotum tinged with pale testaceous. Head brown, with five short blunt yellowish spines, the median erect and the others porrect. Antennae brown, moderately slender; segment I stouter than and nearly twice as long as II; III slender, long; IV wanting. Eyes black. Pronotum moderately convex, closely and finely pitted. Carinae sharply raised, with distinct aerolae behind; lateral carinae divaricating anteriorly. Paranota narrow, moderately reflexed, uniseriate; collar raised, reticulate, the front margin truncate.

Elytra moderately narrowed posteriorly, faintly constricted beyond middle. Costal area narrow, uniseriate; subcostal area broader, mostly biseriate, triseriate at widest point; discoidal area elongate, narrowed at both base and apex, widest near middle, slightly tinged with yellow at apex. Sutural area finely reticulate. Wings almost as long as elytra.

Length, 3.00 mm.; width, 0.84 mm.

Holotype (male), Launceston, Tasmania, Aug. 20, 1918. This species is more delicately reticulate than *P. recentis* and lacks the conspicuous color markings.

Leptoypha hospita Drake and Poor

Leptoypha hospita Drake & Poor, Philip. Jour. Sci., LXII, 1937, p. 12.

Allotype, male: Color and form very similar to holotype (female) but a little more slender. Island of Penang, C. F. Baker, type locality.

Eteoneus virtutis Drake and Poor

Eteoneus virtutis Drake & Poor, Philip. Jour. Sci., LXII, 1937, p. 13.

Mt. Makiling, Luzon, Philippine Islands, male and female, collected by C. F. Baker. These specimens bear the same label and were probably collected with the type.

Melandiola similis Hacker

Melandiola similis Hacker, Mem. Queens. Mus., IX, 1927, p. 21.

One specimen, Botany Bay, N. S. Wales, Australia, collected by H. Petersen.

Tingis buddleiae Drake

Tingis buddleiae Drake, Proc. Ent. Soc. Wash., XXXII, 1930, p. 168.

Forty-nine examples, Los Banos, Luzon, Philippine Islands, at an elevation of 50 meters, May 2, 1934, collected on *Buddleia asiatica* by V. J. Madrid.

Belenus dentatus (Fieb.)

Monanthia (Phyllontochila) dentata Fieber, Ent. Monog., 1844, p. 71, Pl. VI, figs. 2-4.

Belenus dentatus Distant, Ann. Soc. Ent. Belg., LIII, 1909, p. 116.

Los Banos, Luzon, Philippine Islands, female, collected by F. Muir, and 1 specimen collected July 10, 1932 by S. P. Capco, at an elevation of 5 meters; and at Mt. Makiling, Luzon, P. I., C. F. Baker.

Phyllontocheila philippinensis Distant

Phyllontocheila philippinensis Distant, Ann. Mag. Nat. Hist., IX, 1902, p. 355.

Los Banos, Luzon, P. I., 12 specimens, Sept. 15, 1915, C. S. Banks; 2 specimens Sept. 20, 1915, 2 Sept. 29, 1915, 1 Sept. 30, 1915, 1 Oct. 11, 1915, collected by A. Lipayon; 2 specimens Aug. 9, 1915, A. L. Leodoro; and 3 specimens Oct. 24, 1931, by M. de la Cruz.

Hormisdas vicarius Drake

Hormisdas vicarius Drake, Philip. Jour. Sci., XXXII, 1927, p. 56.

Betis, Pampanga, Philippine Islands, Nov. 28, 1928, 16 specimens collected by L. B. Uichanco on *Urena lobata* L. var. *sinuata* (L.) (Malvaceae).

Stephanitis nitoris Drake and Poor

Stephanitis nitoris Drake and Poor, Philip. Jour. Sci., LXII, 1937, p. 17.

Mt. Makiling, Luzon, Philippine Islands, 1 example, C. F. Baker (same label as type); Los Banos, Luzon, P. I., Sept. 26, 1926, 5 specimens, taken by S. M. Cendana; 15 specimens, Feb. 20, 1935, V. J. Madrid, on *Uvaria rufa*.

Stephanitis globulifera Matsumura

Tingis globulifera Matsumura, Senchu-Zukai, II, 1905, p. 36, Tab. 19, fig. 16.

Stephanitis globulifera Horvath, Ann. Nat. Mus. Hung., X, 1912, pp. 321, 330.

Tokyo, Japan, May 30, 1931, 8 specimens taken by L. Gressitt.

Stephanitis nashi Esaki and Takeya

Stephanitis nashi Esaki & Takeya, Mushi, IV, 1931, p. 54.

Tokyo, Japan, May 17, 1931, several specimens, L. Gressitt; Canton, China, 1 example. This species infests the pear tree.

Stephanitis fasciicarina Takeya

Stephanitis (Stephanitis) fasciicarina Takeya, Mushi, IV, 1931, pp. 70-72, Tab. 7, fig. 2, Tab. 8, fig. 6.

Moji, Japan, Sept. 11, 1906, and Tokyo, Japan, Sept. 25, 1915. This species is very closely allied to *S. querci* Bergroth and differs only slightly from it in having a smaller and lower hood and less elevated median carina. The costal area of elytra usually has one less row of areolae in

its widest part. A series of *S. querci* from the Philippines shows some variation in these characters but they do not seem to integrate with paratypes and a series of other specimens of *fasciicarina* from Japan.

Stephanitis subfasciata Horvath

Stephanitis subfasciata Horvath, Ann. Mus. Nat. Hung., X, 1912, p. 325.

Pekalongan, Java, May, 1901, 1 example, F. Muir, collector.

Stephanitis typica Distant

Cadamustus typicus Distant, Ann. Soc. Ent. Belg., XLVII, 1903, p. 47; Fauna Brit. Ind., Rynch., II, 1904, p. 132, fig. 95.

Stephanitis typica Horvath, Ann. Mus. Nat. Hung., X, 1912, p. 325.

Los Banos, Luzon, Philippine Islands, Feb. 26, 1919, numerous specimens, collected on *Anona muricata* Linn., L. B. Uichanco; numerous other specimens from Los Banos by various collectors; three specimens from Butuan, Agusan, P. I., Apr. 22, 1932, L. B. Uichanco. This is one of the most common and most widely distributed tingitids in the Philippine Archipelago.

THE RHYNCHOPHORA OF IOWA

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In 1876, Mr. J. D. Putnam published his "List of Coleoptera found in the vicinity of Davenport, Iowa," "Coleoptera collected at Monticello, Iowa, June 12, 1872," and "Coleoptera collected near Frederick, Monroe Co., Iowa, August, 1869." Mr. H. F. Wickham, in 1888, published "A list of the Coleoptera of Iowa City and vicinity." In 1895, a supplement to this list was published. In 1911, Wickham published "A List of the Coleoptera of Iowa" in which are found 221 species of Rhynchophora reported from various collections and records in literature.

The chief problem of this study has been the determination of specimens found in the Iowa State College and Iowa Insect Survey collections. With Mr. Wickham's 1911 list as a working basis, the project was begun two years ago of bringing together all the records published by the various authors dealing with Iowa species. As the work progressed, it became evident that numerous new records were present in the material studied. All available published records have been carefully checked, and every determination, new or old, was compared with material in the National Museum at Washington by the author in December, 1936.

Over 10,000 specimens, comprising nearly 2000 individual county distribution records, have been studied in the various collections. Four hundred sixty-two species have been definitely shown to occur within the state, while published records of 48 others from surrounding states tend to indicate their probable occurrence within our borders.

The county records are listed under each species. The genera and species are arranged according to Leng's "Catalogue of the Coleoptera of America, north of Mexico." Corrections in a number of the original citations given by Leng have been made. Synonymy has been included in a few cases upon verification by Mr. L. L. Buchanan.

LIST OF IOWA RHYNCHOPHORA WITH DISTRIBUTIONAL DATA

Family Brentidae

Eupsalis minuta Drury. Exot. Ins., 1, 1770, p. 90, t. 42, f. 3, 7. (nec 1837, 1773, p. 95 auct.)

County records: Des Moines, Henry, Jefferson, Johnson, Kossuth, Lee, Linn, Monroe, Story, Van Buren, Wapello.

¹The author takes this opportunity of acknowledging the special debt to Dr. H. H. Knight, under whose direction and aid this project has been pursued; to Dr. Hendrickson, whose prairie insect material has produced a number of very fine records which are included in this work; to Drs. Drake, Harris and Wellhouse for their kind help and interest in the problem; to Mr. H. E. Jaques, whose unselfish co-operation made the Iowa State Insect Survey records available for study; and to Mr. L. L. Buchanan of the United States National Museum whose aid in determining the species of Apion, Curculio, and Cryptorhynchus made their study the more valuable.

Eupsalis minuta var. **lecontei** Power. Ann. Soc. Ent. Fr., 1878, p. 494.

County records: Henry, Story, Worth.

Recognition of this variety is hardly necessary since the male beak is extremely variable in this species. The larger males necessarily tend to have enlarged beaks.

Family Platystomidae

Ormiscus saltator Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 397.

County records: Dickinson, Johnson.

The Dickinson specimens were collected by Mr. L. L. Buchanan and are now in the U. S. National Museum collection. It has been rarely collected.

Eurymycter fasciatus. (Olivier). Entomologie, 4, 1795, p. 9.

County records: Clayton, Davis, Muscatine, Story.

Allandrus bifasciatus Leconte. Proc. Am. Phil. Soc., 1876, p. 396.

County records: Johnson.

Two specimens were collected at Iowa City by Mr. Buchanan. A new state record.

Piezocorynus moestus Leconte. Ann. Lyc. Nat. Hist. N. Y., 1, 1824, p. 172.

County records: Johnson.

Wickham ('11) reported this species from Iowa City, thereby indicating that the specimens were in his personal collection. However, no specimens from Iowa were seen by the author in the Wickham collection which is now deposited in the National Museum. In fact, no specimens of the genus has been seen with an Iowa label. This record, from the distribution indicated in Leng ('20), could be more logically Leconte's *P. mixtus* or Gyllenhal's *P. dispar*.

Euparius marmoreus (Olivier). Entomologie., 3, 1795, p. 12.

County records: Appanoose, Buena Vista, Davis, Des Moines, Dickinson, Henry, Jasper, Jefferson, Lee, Louisa, Monroe, Page, Story, Wapello, Washington, Wright.

In 1888, C. A. Gillette took the species at lights and Raymond, in 1890, took them from milkweed. Wickham denoted its distribution as general throughout the state.

Brachytarsus alternatus (Say). Jour. Acad. Nat. Sci., 5, 1826, p. 250.

County records: Clayton, Davis, Dickinson, Henry, Louisa, Plymouth, Washington.

Brachytarsus sticticus Boheman. Schoenh. Gen. Curc., 1, 1833, p. 172.

County records: Boone, Bremer, Buena Vista, Clayton, Des Moines, Dubuque, Henry, Iowa, Jackson, Jasper, Jefferson, Johnson, Lee, Louisa, Polk, Pottawattamie, Story, Van Buren.

This is the *B. variegatus* Say reported from Iowa by Wickham ('11). Common throughout the state.

Brachytarsus tomentosus (Say). Jour. Phil. Acad. Sci., 5, 1826, p. 251.

County records: Cass, Cherokee, Davis, Decatur, Des Moines, Dickinson, Emmet, Henry, Jackson, Keokuk, Kossuth, Linn, Osceola, Plymouth, Worth.

Brachytarsus paululus Casey. Col. N. A., 1884, p. 194.

County records: Benton, Cass, Cherokee, Dickinson, Henry, Jackson, Mahaska, Plymouth, Worth.

This species was determined almost exclusively from size since its vestiture is almost identical with *B. tomentosus* Say. The two specimens from Cass County, collected by the author, are 1.4 mm. in length, the other specimens are about 1.8 mm. The vestiture seems a little more dense, which, together with the size difference, provides the only distinguishing character. It is probable that biological study will prove its synonymy with *B. tomentosus*.

Brachytarsus limbatus (Say). Jour. Phil. Acad. Sci., 5, 1826, p. 250.

County records: Dickinson, Johnson.

The specimens are in the U. S. National Museum, those from Dickinson having been collected in 1935 by Gould Warren, the Johnson county specimens by Mr. Buchanan in 1916 and 1917.

Brachytarsus plumbeus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 406.

County records: Johnson.

Reported from Iowa City by Wickham ('11). No specimens have been seen from Iowa which are densely clothed with ashgray pubescence, not mottled, and measuring 3.2 mm.

Family Belidae

Ithycerus noveboracensis (Forst.). Nov. Spec. Ins., 1771, p. 35.

County records: Bremer, Clayton, Hancock, Henry, Johnson, Marshall, Polk, Story.

This is the "New York Weevil" of economic literature and is one of the largest curculionids occurring in the state. Ewing's specimen from Marshall County was taken from hickory. The Polk County specimen carries a label notation "5/24/79 from Polk Co. injuring apple. C. F. Clarkson."

Family Curculionidae

Eugnamptus striatus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 5.

County records: Des Moines.

One specimen with the label "Burlington, Ia." was kindly sent from the Kansas University collection by Mr. Lyman Henderson. The striae are large and approximate, the intervals convex throughout the elytra and distinctly less wide than the punctures. It is quite probably this species in spite of its occurrence so far outside of the previously recorded type locality, Florida.

Eugnamptus collaris (Fabricius). Syst. Eleuth., 2, 1801, p. 410.

County records: Cedar, Dickinson, Hancock, Henry, Jackson, Johnson, Jones, Kossuth, Story.

Eugnamptus collaris var. **nigripes** Pierce. Proc. U. S. Nat. Mus., 55, 1913, p. 367.

County records: Story.

A specimen collected June 28, 1892, was taken "On *Quercus alba*." A specimen from the Wickham collection with an Iowa label is in the National Museum.

Eugnamptus collaris var. **fuscipes** Pierce. Proc. U. S. Nat. Mus., 55, 1913, p. 367.

County records: Boone, Hancock, Johnson, Kossuth, Lee.

Two specimens from Lee County were collected from sycamore by Harris and Johnston.

Eugnamptus collaris var. **ruficeps** Pierce. Proc. U. S. Nat. Mus., 55, 1913, p. 367.

County records: Johnson, Story.

These two records disprove Blatchley's assumption that "This variety enjoys a limited southern distribution and, consequently, it should be elevated to the rank of a species." There are no structural differences.

Eugnamptus sulcifrons Gyllenhal. Schoenh. Curc., 5, (1), 1839, p. 343.

County records: Boone, Linn, Madison, Polk, Story.

These specimens exhibited a distinct frontal sulcus, but otherwise look very much like *E. collaris*.

Eugnamptus angustatus (Herbst). Kafer, 7, 1797, p. 140.

County records: Johnson, Linn.

Reported from these two localities by Wickham ('11). No specimens were seen in the National Museum.

Rhynchites bicolor (Fabricius). Syst. Ent., 1775, p. 131 (nec 98-388, Leng).

County records: Adair, Allamakee, Blackhawk, Bremer, Buena Vista, Cass, Cherokee, Clay, Crawford, Dickinson, Franklin, Green, Hamilton, Henry, Ida, Iowa, Lee, Linn, Lyon, Monona, Muscatine, Osceola, Plymouth, Story, Union, Washington, Winnebago, Woodbury, Worth.

This species, the "Rose Weevil," is common on wild rose throughout the state.

Rhynchites aeneus Boheman. Nov. Col. Spec., 1829, p. 22.

County records: Dickinson, Plymouth, Pottawattamie, Story, Woodbury.

Found by Hendrickson ('28) on the stem of Resin weed (*Silphium laciniatum* L.) and a pair mating on *Brauneria purpurea* Britton.

Rhynchites hirtus (Fabricius). Syst. Eleuth., 1801, p. 421.

County records: Muscatine.

A single specimen was collected by Mr. Denning on the above date in a wooded area near Muscatine, Iowa. The closest previous record seems to be from Michigan.

Rhynchites cyanellus Leconte. Proc. Am. Phil. Soc. 15, 1876, p. 8.

One specimen with the label "Ia." from the collection of C. V. Riley deposited in the U. S. National Museum. Harrington ('91) reported it feeding and copulating on willow.

Rhynchites aeratus Say. Curc., 1831, p. 3.

County records: Dickinson, Woodbury.

Eight specimens of this species, seven of which were taken in Dickinson County by Mr. Buchanan, are in the U. S. N. M. collection.

Attelabus analis Illiger. N. Mag. Lieb. Ent. (Schneider's), 5, 1794, p. 616.

County records: Johnson.

Pierce ('13) reports it from a Wickham specimen.

Attelabus nigripes Leconte. Ann. Lyc. Nat. Hist. N. Y., 1, (6), 1824, p. 171.

County records: Dickinson, Henry, Johnson, Monroe.

Might be confused with a small *Rhynchites bicolor*.

Attelabus pubescens Say. Jour. Phil. Acad. Nat. Sci., 5, 1826, p. 252.

County records: Clayton, Crawford, Johnson, Story.

Reported by Wickham ('11) under the name *A. rhois* Boh., a synonym.

Pterocolus ovatus (Fabricius). Syst. Eleuth., 2, 1801, p. 426.

County records: Johnson, Dickinson.

A nice series of this peculiar weevil which Mr. Buchanan collected at Lake Okoboji, Ia., are now in the National Museum collection. He found them feeding on oak sprouts.

Apion erraticum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 44.

County records: Johnson.

Reported by Wickham ('11).

Apion impunctistriatum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 48.

County records: Cherokee, Clinton, Decatur, Dickinson, Henry, Johnson, Jones, Muscatine, Plymouth, Sioux, Story, Washington.

Apion melanarium Gerstaecker. Stett. Ent. Zeit., 15, 1854, p. 261.

County records: Bremer, Buchanan, Cherokee, Clarke, Des Moines, Dickinson, Emmet, Harrison, Henry, Jackson, Keokuk, Lee, Louisa, Plymouth, Polk, Story, Van Buren, Wapello, Washington, Winnebago, Worth.

Apion sp. 1.

A specimen in the National Museum collected at Lake Okoboji, Iowa, August 22, 1916, by Mr. L. L. Buchanan has been placed by him near *virile*. This is one of nearly a dozen undetermined species of *Apion* from Iowa. Mr. Buchanan is planning to monograph the genus in the near future and these species will be fully considered at that time.

Apion robustum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 45.

County records: Davis, Dickinson, Harrison, Johnson, Jones, Linn, Sioux.

Apion pennsylvanicum Boheman. Schoenh. Curc., 5, (1), 1839, p. 417.

County records: Lee, Johnson, Story, Washington.

Apion sp. 2.

A specimen collected by H. C. Knutson in Johnson County, June 20, 1934, has been placed by Mr. Buchanan near *A. pennsylvanicum*.

Apion occidentale Fall. Trans. Am. Ent. Soc., 25, 1898, p. 126.

County records: Cherokee, Dickinson, Plymouth.

Apion sp. 3.

Fourteen specimens of Iowa material are grouped with a specimen notation "near *occidentale*." It is possible that two species are present in this material.

Apion punctinatum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 46.

County records: Dickinson.

First recorded from the state by Buchanan ('22).

Apion tenuirostrum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 62.

County records: Dickinson, Story, Wright.

Buchanan ('22) records it from Dickinson and Wright counties.

Apion sp. 4.

A specimen from Lake Okoboji, July 22, 1916 collected with two others by Buchanan are noted to have "simple claws; no mucro on anterior tibia; near *tenuirostrum*."

Apion smithi Wagner. Deutsche Ent. Zeitschr., 1909, p. 767.

County records: Henry, Washington.

Apion modestum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 58.

County records: Dickinson, Henry.

This is a homonym of *A. modestum* Kirby, 1817, p. 234, which Wagner ('10) considers, in turn, a synonym of *A. loti* Kirby, 1808, p. 58.

Apion walshi Smith. Trans. Am. Ent. Soc., 11, 1884, p. 57.

County records: Johnson.

Apion minor Smith. Trans. Am. Ent. Soc., 11, 1884, p. 56.

County records: Dickinson, Johnson, Story.

Apion turbulentum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 56.

County records: Dickinson, Story.

Apion griseum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 59.

County records: Bremer, Buchanan, Cedar, Des Moines, Dickinson, Henry, Johnson, Lee, Louisa, Monroe, Muscatine, Story, Van Buren.

Seemingly common throughout the eastern half of the state.

Apion porcatum Boheman. Schoenh. Curec., 5, (1), 1839, p. 374.

County records: Des Moines, Lee.

Apion centrale Fall. Trans. Am. Ent. Soc., 25, 1898, p. 151.

County records: Dickinson, Johnson, Palo Alto, Story.
Buchanan ('22) first records the species from Iowa.

Apion rostrum Say. Jour. Acad. Nat. Sci., Phila., 5, 1826, p. 253.

County records: Story, Taylor.
Infests the seed pods of Baptisia in Iowa.

Apion nigrum Herbst. Kafer, 7, 1797, p. 132 (nec p. 122, Leng).

County records: Story.
Reported by Wickham ('11).

Apion varicorne Smith. Trans. Am. Ent. Soc., 11, 1884, p. 60.

County records: Boone, Clinton, Dickinson, Humboldt, Lyon, Plymouth, Van Buren, Winnebago.
Reported by Buchanan ('22) for the first time.

Apion decoloratum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 52.

County records: Henry, Johnson, Story.
Fall ('98) reports it on Desmodium at Iowa City. Found in the seed pods of Meibomia.

Apion emaciipes Fall. Trans. Am. Ent. Soc., 25, 1898, p. 166.

County records: Delaware.
A new state record.

Apion carinatum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 52.

County records: Dickinson.
Recorded by Buchanan ('22).

Apion attenuatum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 62.

County records: Plymouth, Story, Winneshiek.
A new state record.

Apion notabile Buchanan. Proc. Ent. Soc. Wash., 24, 1922, p. 83.

County records: Dickinson.
A note to be found on the pin states that it is "apparently equal to *spinipes* Fall (L. B. '32)."

Apion commodum Fall. Trans. Am. Ent. Soc., 25, 1898, p. 154.

One specimen labelled as this species is in the synoptic collection at the National Museum.

Apion sp. 5.

A specimen with the note "(runs to *commodum*)" which was collected by Mr. Buchanan at Lake Okoboji, July 24, 1916, was separated from the rest of the *commodum* specimens.

Apion sp. 6.

A specimen bearing the note (near *turbulentum*)" is in the National Museum collection. It was collected by Mr. Buchanan at Lake Okoboji, July 16, 1917.

Apion sp. 7.

This specimen from Washington, Iowa, was collected by Dr. Hendrickson and listed in his 1930 work as *Apion* sp. 1. It was again checked by Mr. Buchanan for the author, and the additional label states "Not found in N. W. coll." It is probably a new species.

Apion sp. 8

Two specimens, including Hendrickson's ('30) sp. 2 and 4, have been rechecked by Mr. Buchanan who finds nothing comparable in the N. M. collection.

Apion sp. 9.

One specimen, perhaps from the series designated as sp. 3 by Hendrickson ('30), carries the notation on the pin, "*Apion* sp. (claws virtually simple)." It is in the N. M. collection.

Apion sp. 10.

Four specimens which were collected Sept. 27, 1893, have the accession catalog number "889" on each pin. Referring to this number, one finds that the specimens were collected "9/27/93 by F. A. Sirrine, I. A. C. (Iowa Agricultural College), taken from seeds of *Dalea alopecuroides*." Belongs to group III.

Phyxelis rigidus (Say). Curc., 1831, p. 11 (nec p. 12, Leng).

County records: Clayton, Delaware, Fayette, Floyd, Hancock, Henry, Howard, Johnson, Kossuth, Mahaska, O'Brien, Palo Alto, Story, Washington, Winneshiek.

Undoubtedly very common throughout the state. Occasionally reported attacking potatoes in Iowa.

Anametis granulata (Say). Curc., 1831, p. 12.

County records: Buchanan, Cerro Gordo, Clayton, Dickinson, Fremont, Greene, Hamilton, Henry, Iowa, Jackson, Johnson, Jones, Kossuth, Louisa, Plymouth, Sioux, Story, Webster, Winnebago. Common throughout the state.

Panscopus maculosus Blatchley. Rhynch. N. E. U. S., 1916, p. 105.

County records: Story.

Collected on Wahoo (*Evonymus atropurpureus* Jacq.) at Ames, May 25, 1934, by Mr. Hansberry.

Panscopus aequalis (Horn). Proc. Am. Phil. Soc., 15, 1876, p. 55.

County records: Johnson, Story.

Reported from Ames and Iowa City by Wickham ('11). These records are probably of the preceding species although it is reported from Kansas.

Aracanthus pallidus Say. Curc., 1831, p. 9.

Reported from Iowa by Leng ('20). No Iowa specimens have been seen by the author.

Tanymecus confusus Say. Curc., 1831, p. 9.

County records: Appanoose, Boone, Bremer, Calhoun, Cherokee, Clay, Clayton, Davis, Des Moines, Henry, Iowa, Jackson, Jefferson, Jones, Louisa, Muscatine, Plymouth, Sioux, Story, Van Buren, Wapello, Winneshiek.

Very common throughout the state.

Pandeleiteius hilaris (Herbst). Kafer, 7, 1797, p. 58.

County records: Appanoose, Clayton, Johnson, Linn, Louisa, Story.

Hormorus undulatus (Uhler). Proc. Acad. Nat. Sci., Phila., 7, 1855, p. 416.

County records: Delaware, Hancock, Story.

Graphorinus vadosus Say. Curc., 1831, p. 8.

County records: Sioux.

Hendrickson's ('30) record remains unique for the state. It resembles a great deal the following species.

Epicaerus imbricatus Say. Jour. Acad. Nat. Sci. Phila., 3, 1824, p. 317.

County records: Blackhawk, Cherokee, Dallas, Davis, Des Moines, Dickinson, Dubuque, Fayette, Hardin, Henry, Iowa, Lee, Monroe, Muscatine, Plymouth, Ringgold, Sioux, Story, Union, Washington, Woodbury.

Walsh ('64) reports that "it infests apple and cherry trees and gooseberry bushes in Iowa." Common throughout the state. It is the "imbricated snout beetle" of literature.

Pantomorus godmani (Crotch). Proc. Zool. Soc. Lond., 1867, p. 389.

County records: Johnson, Story.

Commonly referred to as "Fuller's Rose Weevil."

Pantomorus tessellatus (Say). Jour. Acad. Nat. Sci. Phila., 3, 1824, p. 318.

County records: Lee, Pottawattamie.

Two specimens from Lee county and Pottawattamie county are less densely squamose than another specimen from Lee county, but all are

clothed with silver white scales and present a much more robust appearance throughout than the specimen of *P. godmani*, which is brownish in coloration and with the thorax much narrower behind.

Pantomorus tessellatus* var. *pallidus (Horn). Proc. Am. Phil. Soc., 15, 1876, p. 94.

County records: Pottawattamie.

This specimen was undoubtedly collected at the same time that the above specimen was taken, and resembles the more dense specimen from Lee county mentioned above. It is determined as such in the National Museum collection.

Lepidocricus minor (Buch.) Proc. U. S. N. M., 76, 1929, p. 6.

County records: Hamilton, Iowa, Johnson, Keokuk, Story.

The Johnson record is that of Wickham ('11) for *L. herricki*. According to Buchanan ('29), *herricki* enjoys a more southern distribution.

Lepidocricus oblongus (Buch.) Proc. U. S. N. M., 76, 1929, p. 8.

County records: Dickinson, Osceola, Palo Alto, Story.

Perhaps only a variety of the preceding.

Barypeithes pellucidus (Boheman). Schoenh. Curc., 2, 1834, p. 507.

County records: Story.

A new state record.

Otiorrhynchus* (Tournieria) *ovatus (L.). Syst. Nat., 1758, p. 384.

County records: Allamakee, Boone, Bremer, Butler, Cerro Gordo, Clayton, Emmet, Des Moines, Dickinson, Floyd, Hancock, Henry, Jackson, Johnson, Kossuth, Linn, Story, Winneshiek.

Known as the "Strawberry crown-girdler."

Cercopeus chrysorrhoeus (Say). Curc., 1831, p. 13.

County records: Johnson, Story.

Aphrastus taeniatus Say. Curc., 1831, p. 9.

County records: Blackhawk, Boone, Fremont, Henry, Iowa, Johnson, Story.

Sitona flavescens (Marsham). Ent. Brit. etc., 1802, p. 311.

County records: Boone, Franklin, Kossuth, Pocahontas, Story.

This is the "yellow clover curculio".

Sitona hispidulus (Fabricius). Gen. Ins., 1777, p. 226.

County records: Boone, Butler, Delaware, Des Moines, Fayette, Henry, Jefferson, Kossuth, Lee, Louisa, Lucas, Mills, Scott, Story, Union, Washington, Wapello.

Known as the "clover root curculio." A new state record.

Sitona lineellus Bonsdorff. Hist. Nat. Curc. Suec., 1785, p. 30.

County records: Boone, Kossuth, Lucas, Winnebago.

Recorded from Kossuth and Winnebago by Hendrickson ('30).

Sitona scissifrons Say. Curc., 1831, p. 10.

County records: Buchanan, Dickinson, Hamilton, Hancock, Kossuth, Osceola, Palo Alto, Plymouth, Story, Winneshiek, Woodbury, Wright.

Sitona scissifrons Say, according to Mr. Buchanan, is the name to be given to this species instead of *S. tibialis* Hbst.

Hypera (Antidonus) punctata (Fabricius). Syst. Ent., 1775, p. 150.

This species is so common throughout the state on clover that little needs to be said of it. Known as the "clover-leaf beetle", it has been a serious pest in certain years past. It was not numerous during the extremely dry seasons of 1935 and 1936.

Hypera eximius (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 414.

Common on *Rumex* throughout the state. Pupating cocoons were collected at Le Mars, Iowa on June 13, 1936, the adults emerging on June 18, 1936. Not reported by Wickham ('11), but recorded from Iowa by Titus ('11).

Hypera (Eirrhinomorphus) rumicis (Linn.). Syst. Nat., 1758, p. 379.

Buchanan ('23) reported this weevil from Iowa City where it had been collected May, 1917 from *Polygonum*. Its distribution seems to be identical with the above species, and, aside from the difference in color pattern, it is identical with *H. eximius*.

Hypera (Eirrhinomorphus) comptus (Say). Curc., 1831, p. 12.

County records: Dubuque, Jefferson, Lee.

Reported from Iowa by Titus ('11). Csiki ('34) considers it a variety of *rumicis* L. The type of the scale makes this conclusion impossible.

Hypera trivittatus (Say). Curc., 1831, p. 12.

County records: Story.

One specimen, badly denuded, with the scales deeply emarginate but not cleft as in *nigrirostris*, seems to fit the description of this species.

Hypera (Dapalinus) nigrirostris (Fabricius). Syst. Ent., 1775, p. 132.

County records: Common throughout the state.

Listronotus sordidus (Gyllenhal). Schoenh. Curc., 2, (1), 1834, p. 280.

County records: Story.

Known only from the published records of Wickham ('11).

Listronotus tuberosus Leconte. Proc. Phil. Acad. Sci., 1876, p. 130.

County records: Johnson, Story.

The specimen from Story county has been the only one of this distinct species seen by the author. On *Sagittaria* and *Carex*.

Listronotus squamiger (Say). Curc., 1831, p. 11.

County records: Jackson, Story.

Listronotus inaequalipennis (Boheman). Schoenh. Curc., 6, 1842, p. 189, (nec 1840, Leng).

County records: Des Moines.

Known only from the Wickham ('11) records. Henderson (i. litt.) places it as a synonym of *squamiger*.

Listronotus caudatus (Say). Jour. Acad. Sci., 3, 1824, p. 311 (nec 1823, Leng).

County records: Cherokee, Dickinson, Franklin, Jackson, Jefferson, Johnson, Story.

Mr. Sooter collected two specimens from *Scirpus* sp. at Lost Island, Ruthven, Iowa.

Listronotus appendiculatus (Boheman). Schoenh. Curc., 6, 1842, p. 192 (nec 1840, Leng).

County records: Johnson, Muscatine, Story.

Listronotus nebulosus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 133.

County records: Linn, Story.

Listronotus frontalis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 133.

County records: Linn, Story.

Listronotus latiusculus (Boheman). Schoenh. Cur., 1842, p. 199 (nec 1840, Leng).

County records: Blackhawk, Des Moines, Dickinson, Johnson, Louisa, Mahaska, Story, Winneshiek.

Harris ('26) discusses this species as a pest of carrots at Burlington, Iowa, during the summer of 1925.

Hyperodes solutus (Boheman). Schoenh. Curc., 6, (2), 1842, p. 197 (nec 1840, Leng).

County records: Bremer, Clayton, Cherokee, Decatur, Dickinson, Henry, Iowa, Jackson, Jefferson, Johnson, Lee, Mitchell, Osceola, Pocahontas, Story, Winnebago.

Swept in numbers from swamps and slough vegetation.

Hyperodes grypidioides (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 35.

County records: Polk, Story.

Hyperodes indistinctus (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 32.

County records: Johnson, Linn.

Blatchley ('16) considers this species synonymous with *H. solutus*.

Hyperodes interpunctatulus (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 36.

County records: Lee, Story.

Hyperodes ulkei (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 37.

County records: Polk, Story.

Hyperodes tenebrosus (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 38.

County records Decatur, Dickinson, Keokuk, Plymouth, Story.

Hyperodes montanus (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 39.

County records: Bremer, Dickinson, Guthrie, Lee, Story.

Hyperodes delumbis (Gyllenhal). Schoenh. Curc., 1834, p. 283 (nec 34-203, Leng).

County records: Bremer, Cass, Delaware, Fayette, Henry, Linn, Osceola, Polk, Story, Wapello.

Undoubtedly the most common species of the genus in the state. Flies to the lights during the spring and summer in numbers.

Hyperodes sparsus (Say). Curc., 1831, p. 11.

County records: Allamakee, Boone, Bremer, Dickinson, Floyd, Fremont, Henry, Jefferson, Kossuth, Lee, Louisa, Lucas, Story, Van Buren, Washington, Worth.

Hyperodes obscurellus (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 45.

County records: Kossuth.

Determined by Mr. Buchanan for Hendrickson ('30).

Hyperodes echinatus (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 46.

County records: Clayton, Story.

One specimen with an Ames, Iowa label notes that it is a "Purslane leaf-miner."

Hyperodes vitticollis (Kirby). Ins. Rich. Bor. Amer., 4, 1837, p. 200.

County records: Bremer, Johnson.

The Bremer county record is in the Casey collection.

Hyperodes humilis (Gyllenhal). Schoenh. Curc., 2, (1), 1834, p. 284.

County records: Plymouth, Story.

Hyperodes porcellus (Say). Curc., 1831, p. 11.

County records: Jefferson, Story.

Hyperodes sp. 1.

Two specimens, one from Ames, Iowa and the other from Montrose, Iowa are included here, and should be placed under group one with those having the second funicular joint much longer than the first.

Hyperodes sp. 2.

One specimen collected by the author at Ames, Iowa, last May 21, runs to "dorsalis," but there are conflicting characteristics which make its definite determination impossible.

Hyperodes sp. 3.

One specimen collected May 31 at Lost Island Park, Ruthven, Iowa, by the author posses a striking resemblance to the female "*delumbis*" speci-

mens. However, each interval of the elytra has long setae, whereas, in *delumbis* specimens, only the alternate intervals are with setae.

Hyperodes sp. 4.

A series of six specimens of this striking species include Appanoose, Cass, Dickinson, Story (2), and Winneshiek counties. It is large enough for a *Listronotus*, but the second funicular joint is but little longer than the first, a character that would tend to place it in the first group of the *Hyperodes*.

Hyperodes sp. 5.

An Ames specimen collected by Mr. Glawe on June 29, 1930, does not seem to key to any of the known *Hyperodes* species. It looks much like a minute *Listronotus* except for the subequal first joints of the funicle. Mr. Henderson, who is monographing the genus, thinks it may be a new species.

Hyperodes sp. 6.

A specimen from the collection of Dr. Harris bears an Ames label and was collected June 21, 1928. It has the general appearance of *H. sparsus* and *H. delumbis* combined. The thorax gives an appearance of being granulated, the elytral intervals each having a row of short, clubbed setae, and the hind tibia clothed on the inner surface with long silken hairs.

The six species listed above have occupied the study of the author for long periods of time, and seemingly without tangible conclusions. Undoubtedly, Mr. Henderson's work on the genus will provide the necessary explanations.

Hylobius pales (Herbst). Nat. Ins. Kaf., 7, 1797, p. 31, t. 99, f. 10. (nec Boh. 34-340, Leng.)

County records: Story.

A unique addition to the state weevil list collected by the author along the Skunk river south of Ames.

Euclyptus ferrugineus (Leconte). Proc. Phil. Soc. Am., 15, 1876, p. 174.

County records: Johnson.

No specimens were seen by the author in the collections studied. The species measures but 1.5 mm., which perhaps accounts for its rarity.

Dorytomus mucidus (Say). Curc., 1831, p. 14.

County records: Henry, Johnson, Pottawattamie, Scott, Story, Van Buren.

Swept from willow in early spring.

Dorytomus laticollis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 164.

County records: Johnson, Story.

Dorytomus parvicollis Csy. Vol. 4, Coleop. Mem., 1892, p. 367.

County records: Story, Winneshiek.

It is doubtful if the more strongly toothed femora is sufficient for a specific differentiation.

Dorytomus indifferens Casey. Col. Not., 4, 1892, p. 375.

County records: Story, Boone.

Dorytomus fusciceps Casey. Col. Not., 4, 1892, p. 377.

County records: Lee (?).

The paratype (U.S.N.M. Paratype 36694) is labelled "Iowa." It is unlike any specimens of the preceding species.

Dorytomus brevicollis Lec. Proc. Am. Phil. Soc., 15, 1876, p. 165.

County records: Story.

Dorytomus squamosus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 166.

County records: Story, Van Buren.

Recorded by Casey ('92) from Iowa.

Grypidius equiseti (Fabricius). Syst. Ent., 1775, p. 130 (nec 98-403, Leng).

County records: Johnson, Story.

Notaris puncticollis (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 163.

County records: Dickinson, Story.

Notaris bimaculatus (Fabricius). Mant. Ins., 1, 1787, p. 98.

County records: Dickinson, Story, Winnebago.

Reported by L. L. Buchanan from Spirit Lake and Lake Okoboji, Iowa in 1923.

Pachyphanes discoideus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 169.

County records: Henry, Iowa, Johnson, Story, Winnebago, Winneshek.

Pachyphanes amoenus (Say). Curc., 1831, p. 26.

County records: Johnson, Story.

Reported by Wickham ('11).

Pachyphanes lineolatus Casey. Coleop. Not., 4, 1892, p. 385.

County records: Lee (?).

A specimen in the Casey cabinets with the labels "Iowa" and "B."

Desmoris scapalis var. **compar** Dietz. Trans. Am. Ent. Soc., 21, 1894, p. 123.

County records: Dickinson, Poweshiek.

Desmoris pervisus Dtz. Trans. Am. Ent. Soc., 21, 1894, p. 125.

County records: Adams, Appanoose, Benton, Cedar, Delaware, Dickinson, Dubuque, Hancock, Story, Union.

Taken on sunflower at Ames by Hendrickson ('30).

Desmoris constrictus (Say). Phil. Ac. Nat. Sci., 4, 1824, p. 313.

County records: Carroll, Cherokee, Dickinson, Iowa, Kossuth, Lucas, Plymouth, Pocahontas, Story, Winneshiek.

Collected in great numbers from *Helianthus* sp. at LeMars, Iowa, and at lights during July.

Desmoris sordidus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 173.

County records: Clinton, Dickinson, Lyon, Poweshiek, Union, Webster.

Desmoris fulvus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 172.

County records: Adair, Cherokee, Clarke, Monroe, Plymouth, Story, Winneshiek.

Suggestive of *Smicronyx vestitus*.

Desmoris flavicans (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 171.

County records: Lee (?).

Reported from Iowa by Casey ('92).

Desmoris fiducialis Casey. Col. Nat., 4, 1892, p. 399.

County records: Lee (?).

A specimen from Iowa (Keokuk?) described by Casey under this name bears such a likeness to *D. fulvus*, that it is doubtful if it is not that species.

Smicronyx squalidus Casey. Col. Not., 4, 1892, p. 407.

County records: Cherokee, Dickinson, Hancock, Johnson, Plymouth, Story, Union, Wayne, Winneshiek.

Smicronyx ovipennis Leconte. Proc. Phil. Soc. Am., 15, 1876, p. 170.

County records: Adams, Johnson, Plymouth.

Smicronyx connivens Casey. Col. Not., 4, 1892, p. 398.

County records: Cherokee, Iowa, Story.

Smicronyx congestus Casey. Col. Not., 4, 1892, p. 401.

County records: Story.

Smicronyx sculpticollis Casey. Col. Not., 4, 1892, p. 403.

County records: Grundy.

The Dietz ('94) records are only from "Iowa."

Smicronyx vestitus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 172.

County records: Cherokee, Des Moines, Osceola, Plymouth, Sac, Story, Winneshiek.

Smicronyx perfidus Dietz. Trans. Am. Ent. Soc., 21, 1894, p. 161.

County records: Cherokee, Plymouth.

Smicronyx maculatus Dietz. Trans. Am. Ent. Soc., 21, 1894, p. 162.

County records: Benton, Union, Winneshiek.

Smicronyx corniculatus (Fahreus). Schoenh. Curc., 7, (2), 1843, p. 309 (nec p. 319, Leng).

County records: Bremer, Cass, Franklin, Henry, Story.

Smicronyx griseus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 171.

County records: Boone.

One specimen in the Harris collection seems to fit this species more closely than any other.

Stenopelmus rufinasus Gyllenhal. Schoenh. Curc., 3, (1), p. 469 (nec 35-409, Leng).

County records: Des Moines, Scott.

A large series of specimens collected by Stoner at Dubuque were examined at the National Museum. This is an aquatic species, and from the records, seems to confine itself to the Mississippi River valley.

Endalus limatulus (Gyllenhal). Schoenh. Curc., 3, (1), 1836, p. 319.

County records: Buchanan, Dickinson, Plymouth, Story.

Mr. Hansberry reports it ovipositing on *Juncus* sp. at Independence, June 19, 1934.

Endalus ovalis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 177.

County records: Dickinson.

Hundreds were sifted from the seeds of the river sedge by Dr. Hayden.

Tanysphyrus lemnae (Paykull). Mon. Curc., 1792, p. 78.

County records: Henry, Johnson.

Onychylis nigrirostris (Boheman). Schoenh. Curc., 7, (2), 1843, p. 184.

County records: Johnson, Linn.

Anchodemus angustus Lec. Proc. Phil. Soc. Am., 15, 1876, p. 181.

County records: Hamilton, Johnson.

Collected by the author under boards along the edge of Goose Lake on Oct. 16, 1936. The long swimmerette hairs on the front tibia are distinctive.

Lixellus filiformis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 182.

County records: Dickinson, Linn.

Lissorhoptrus simplex (Say). Curc., 1831, p. 29.

County records: Boone, Jackson, Linn, Story, Washington, Worth.
This is the "Rice Weevil."

Lissorhoptrus apiculatus (Gyllenhal). Schoenh. Curc., 3, (1), 1836, p. 320.

County records: Dickinson, Wapello.

These specimens are in the U. S. N. M. collection.

Bagous restrictus Lec. Proc. Phil. Soc. Am., 15, 1876, p. 187.

County records: Johnson, Linn, Story.

Bagous americanus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 185.

County records: Story.

Bagous mammillatus (Say). Curc., 1831, p. 28.

County records: Dickinson.

Bagous sellatus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 184.

County records: Story.

This is the *Bagous* sp., at "*Andropogon furcatus*—*Spartina Michauxiana* consocias, June 26, 1926" of Hendrickson ('30).

Bagous planatus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 185.

County records: Henry.

Bagous obliquus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 185.

County records: Dickinson, Johnson.

Bagous transversus Lec. Proc. Phil. Soc. Am., 15, 1876, p. 188.

County records: Des Moines, Dickinson, Henry.

Bagous bituberosus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 188.

County records: Johnson.

Phnigodes setosus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 189.

County records: Story.

Thysanocnemis fraxini Lec. Proc. Am. Phil. Soc., 15, 1876, p. 214.

County records: Jefferson, Johnson, Plymouth, Story.

Thysanocnemis punctata Csy. Syn. Notes, Bal., Tych., 1910, p. 129.

County records: Lee (?).

Described from Iowa by Casey in 1910, this species is perhaps one extreme variation of the above species. It is a single specimen type, as is the case in the majority of Casey's types.

Thysanocnemis bishoffi Blatch. Rhyn. N. E. U. S., 1916, p. 241.

County records: Franklin, Louisa, Plymouth, Story.

Thysanocnemis balaninoides Schffr. Jour. N. Y. Ent. Soc., 1908, p. 217.

County records: Louisa, Story.

Thysanocnemis ocularis Casey. Syn. Notes, Bal., Tych., 1910, p. 129.

County records: Story.

Thysanocnemis helvola Lec. Proc. Am. Phil. Soc., 15, 1876, p. 214.

County records: Plymouth, Story.

Tychius sordidus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 217.

County records: Lee (?).

Recorded by Wickham ('11).

Tychius sordidus var. **nimius** Csy.

County records: Lee (?).

Described from Iowa by Casey ('10).

Tychius tectus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 217.

County records: Dickinson, Lyon, Plymouth, Story, Winnebago, Worth.

Tychius aratus Say. Curc., 1831, p. 26 (nec Gyll. 34-414).

County records: Dickinson, Story.

Myrmex myrmex Herbst. Natursystem, etc., 7, 1797, p. 56.

County records: Clayton, Johnson, Lee.

Myrmex chevrolatii Horn. Proc. Am. Phil. Soc., 13, 1873, p. 450.

County records: Cerro Gordo, Clayton, Dickinson, Johnson, Lee, Linn.

Oopterus perforatus (Horn). Proc. Am. Phil. Soc., 13, 1873, p. 453.

County records: Story.

Magdalis perforata Horn. Proc. Am. Phil. Soc., 13, 1873, p. 453.

County records: Story.

Magdalis austera Fall. Trans. Am. Ent. Soc., 1913, p. 29.

County records: Story.

Magdalis olyra (Herbst). Jablonsky, Nat. Ins., 7, 1797, p. 7.

County records: Johnson, Muscatine, Story.

Magdalis pandura (Say). Curc., 1831, p. 7.

County records: Boone, Clayton, Fayette, Henry, Jasper, Johnson, Story.

Magdalis inconspicua Horn. Proc. Am. Phil. Soc., 13, 1873, p. 456.

Reported by Blatchley ('16) from Iowa.

Magdalis salicis Horn. Proc. Am. Phil. Soc., 13, 1873, p. 435.

County records: Clayton, Henry, Lee, Story.

Magdalis barbata (Say). Curc., 1931, p. 6.

County records: Henry, Tama.

Magdalis armicollis Say Jour. Ac. Nat. Sci., 1824, p. 312.

County records: Boone, Butler, Clayton, Emmet, Fayette, Hancock, Henry, Johnson, Monroe, Sioux, Story, Wineshiek.

This is the "elm weevil" and varies greatly in size and color.

Magdalis armicollis var. **pallida** (Say). Curc., 1831, p. 7.

County records: Johnson, Story.

Curculio caryae (Horn). Proc. Am. Phil. Soc., 13, 1873, p. 460.

County records: Jefferson.

Curculio rectus (Say). *Curc.*, 1831, p. 16.

County records: Cherokee, Howard, Johnson, Story, Winneshiek.

Curculio nasicus (Say) *Curc.*, 1831, p. 16.

County records: Dickinson, Howard, Johnson, Story.

Curculio pardalis (Chttm.). *Proc. Ent. Soc. Wash.*, 10, 1908, p. 25.

County records: Dickinson, Henry, Story.

Curculio confusor (Hamilton). *Desc. n. sp. Col.*, 1893, p. 309.

County records: Story.

The single specimen bears the note "on *Quercus alba*."

Curculio iowensis (Casey). *Can. Ent.*, 42, 1910, p. 122.

County records: Lee.

A species described by Casey from Keokuk, Iowa, the type locality, from a single female specimen. It was reduced to a variety of *confusor* by Leng ('20), but placed as a distinct species by Chittenden ('27).

Curculio baculi (Chitt.). *Proc. Ent. Soc. Wash.*, 10, 1908, p. 20.

County records: Dickinson, Henry, Muscatine, Story.

Curculio obtusus (Blanchard). *Bul. Brook. Ent. Soc.*, 7, 1884, p. 107.

County records: Story.

Curculio strictus (Casey). *Ann. N. Y. Ac. Sci.*, 9, 1897, p. 660.

County records: Dickinson, Harrison, Muscatine, Page, Story.

The determination label by Mr. Buchanan states that there is a possibility that more than one species is present in the above records.

Curculio numenius (Chttm.). *Ent. Amer.*, 8, 1927, p. 178.

County records: Dickinson, Story.

Buchanan's Iowa specimens from Dickinson county were included by Chittenden ('27) in his description of the species.

Tachypterellus quadrigibbus magnus List. *Bull. Colo. Agr. Exp. Sta.* No. 385, 1932, p. 10.

County records: Story.

Anthonomus scutellaris Leconte. *Proc. Ac. N. S. Phila.*, 1858, p. 79.

County records: Story, Johnson.

Anthonomus profundus Leconte. *Proc. Am. Phil. Soc.*, 15, 1876, p. 198.

County records: Henry, Jefferson, Johnson, Story.

Anthonomus nebulosus Lec. *Proc. Am. Phil. Soc.*, 15, 1876, p. 107.

County records: Story.

A new state record.

Anthonomus virgo Dietz. *Trans. Am. Ent. Soc.*, 18, 1891, p. 206.

County records: Iowa.

Hendrickson ('30) records this unique species from Iowa.

Anthonomus haematopus Boheman. Schoenh. Curc., 7, (2), 1843, p. 222.

County records: Dickinson, Johnson, Story, Worth.

Reported from Iowa City under the name of *A. sycophanta* Walsh. *A. bolteri* Dtz., reported also by Wickham, is a variety according to Leng ('20). The author could scarcely detect even that difference in U.S.N.M. material.

Anthonomus suturalis Lec. Ann. Lyc. Nat. Hist. N. Y., 1, 1824, p. 171.

County records: Henry, Iowa, Jefferson, Story.

Anthonomus flavicornis Boheman. Schoenh. Curc., 7, (2), 1843, p. 231.

County records: Henry, Linn.

Anthonomus corvulus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 201.

County records: Johnson.

Reported by Wickham ('11). No specimens were seen.

Anthonomus subguttatus Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 213.

Recorded by Dietz and Wickham.

Anthonomus signatus Say. Curc., 1831, p. 25.

County records: Story.

Anthonomus consimilis Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 216.

A specimen from "Iowa" which was determined by Mr. Dietz for Col. Hubbard & Schaeffer is now in the U.S.N.M. collection.

Anthonomus musculus Say. Curc., 1831, p. 15.

County records: Jefferson, Johnson, Linn, Story.

Anthonomus nigrinus Boheman. Schoenh. Curc., 1843, p. 230.

County records: Page

Anthonomus scutellatus Gyllenhal. Schoenh. Curc., 3, (1), 1836, p. 342.

County records: Johnson, Story.

Anthonomus orchestoides Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 226.

County records: Henry.

Anthonomus squamosus Leconte. Proc. Am. Philos. Soc., 15, 1876, p. 202.

County records: Dickinson, Lyon, O'Brien, Poweshiek, Sioux, Story, Wright.

Anthonomus tectus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 203.

County records: Johnson, Sioux, Story.

Anthonomus molochinus Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 231.

County records: Dickinson.

A series in the U.S.N.M. collection. Looks much like *A. tectus*.

Anthonomus rufipes Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 204.

County records: Adair, Dickinson, Humboldt, Story.

Anthonomus disjunctus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 204.

County records: Dickinson, Henry, Mahaska, Story.

Anthonomus robustulus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 205.

County records: Audubon, Clinton, Dickinson.

Anthonomus unguularis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 206.

County records: Henry.

Anthonomus nubiloides Fall. Bull. Brook. Ent. Soc., 23, 1928, p. 239.

County records: Johnson.

The specimen is labelled *nubilus* Lec. in the U.S.N.M. collection, and is perhaps the record from which Wickham reports that species.

Anthonomus decipiens Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 206.

County records: Bremer, Dickinson, Dubuque, Henry, Jefferson, Johnson, Keokuk, Lee, Story.

Anthonomus ligatus Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 245.

County records: Dickinson.

Anthonomopsis mixtus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 206.

County records: Dickinson, Henry, Johnson, Plymouth, Story, Woodbury.

Pseudanthonomus crataegi (Walsh). Proc. Ent. Soc. Phila., 6, 1867, p. 266.

County records: Henry, Howard, Johnson, Linn, Plymouth, Story.

Pseudanthonomus facetus Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 252.

Reported by Angell ('93) and Wickham ('11).

Chelonychus longipes Dietz. Trans. Am. Ent. Soc., 18, p. 255.

County records: Dickinson, Hancock.

This is the first record of the species in the state.

Elleschus ephippiatus (Say). Curc., 1831, p. 25.

County records: Boone, Bremer, Cerro Gordo, Dickinson, Jefferson, Johnson, Pottawattamie, Story.

Orchestes ephippiatus Say. Curc., 1831, p. 16.

County records: Cherokee, Dickinson, Fremont, Scott, Story.

Orchestes niger Horn. Proc. Am. Phil. Soc., 13, 1873, p. 462.

County records: Dickinson, Muscatine, Story.

Orchestes illinoisensis Fall. Trans. Am. Ent. Soc., 39, 1913, p. 64.

County records: Boone, Lee.

Orchestes canus Horn. Proc. Am. Phil. Soc., 17, 1878, p. 620.

County records: Henry, Johnson, Story.

The Story specimen carries the notation "on apple."

Orchestes mixtus Blatchley. Rhyn. N. E. U. S., 1916, p. 282.

County records: Johnson.

One specimen in the U.S.N.M. collection fits this species.

Orchestes pallicornis Say. Curc., 1831, p. 16.

County records: Boone, Johnson, Story.

Orchestes rufipes Lec. Proc. Am. Phil. Soc., 15, 1876, p. 208.

County records: Dickinson.

These records are in the U.S.N.M. collection.

Acalyptus carpini (Herbst). Ent. Syst., 1, (2), 1792, p. 409.

County records: Howard.

One specimen in the U.S.N.M. collection from Elma, Iowa.

Prionomerus calceatus (Say). Curc., 1831, p. 15.

Four specimens with the label "I." in the I.S.C. collection. One specimen "coll. C. V. Riley," "Iowa" in the U.S.N.M. collection.

Piazorhinus scutellaris (Say). Proc. Ac. N. S. Phila., 1826, p. 252.

County records: Johnson.

Nanophyes pallidulus (Gravenhorst.). Verg. Uber. d. Zool. Syst. Gott., 1807, p. 203.

County records: Johnson.

One specimen in the U.S.N.M. collection from the Wickham material.

Gymnetron tetrum (Fabricius). Ent. Syst., 1, 1792, p. 406 (nec 01-448).

County records: Johnson, Linn, Madison, Pottawattamie, Story.

Common in mullein seeds.

Gymnetron plagiellum Gyllenhal. Schoenh. Curc., 4, (2), 1838, p. 759.

Pierce ('19) notes the species in Iowa.

Gymnetron netum (Germar). Mag. Ent., 4, 1821, p. 312.

County records: Henry, Muscatine, Story.

Determined by Buchanan as this species.

Miarus hispidulus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 221.

County records: Linn, Story.

A label "*on Quercus alba*" is pinned with the Story specimen.

Cleonus plumbeus Lec. roc. hil. Soc. Am., 15, 1876, p. 150.

County records: Story.

Wickham ('11) reports it without definite record.

Cleonus frontalis Lec. Proc. Phil. Soc. Am., 15, 1876, p. 150.

County records: Dickinson, Plymouth.
A new state record.

Lixus caudifer Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 156.

County records: Lee (?).
Probably a Keokuk, Iowa, specimen, now in the Casey collection.

Lixus rubellus Rand. Bost. Jour. N. H., 2, (1), 1838, p. 41.

County records: Dickinson, Palo Alto.
The specimens are in the U.S.N.M. collection.

Lixus marginatus Say Curc., 1831, p. 13.

County records: Bremer, Henry, Lee, Page, Plymouth, Van Buren.

Lixus musculus Say. Curc., 1831, p. 14.

County records: Johnson, Story, Washington, Winneshiek.

Lixus parvus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 157.

County records: Johnson.
Specimen in the U.S.N.M. collection.

Lixus concavus Say. Curc., 1831, p. 14.

County records: Cherokee, Jackson, Johnson, Lee, Plymouth, Story,
Webster.
This is the "rhubarb curculio." Common on *Rumex* spp.

Lixus mucidus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 158.

County records: Cherokee, Iowa, Johnson, Story.

Lixus laramienseis Casey. Ann. N. Y. Acad. Sci., 6, 1891, p. 204.

County records: Story.
This is a *Lixus* sp. 1 of Hendrickson ('30), reported from *Spartina*
consociates.

Lixus sylvius Boheman. Schoenh. Curc., 1843, p. 430 (nec 1844, Leng).

County records: Clayton, Henry, Mahaska, Story.
A new state record.

Lixus scrobicollis Boheman. Schoenh. Curc., 3, (1), 1836, p. 84.

County records: Jefferson, Page.

Lixus fimbriolatus Boheman. Schoenh. Curc., 3, (1), 1836, p. 42 (nec
1826, Leng).

County records: Dickinson, Hancock, Henry, Johnson, Lyon, Mon-
roe, Pottawattamie, Story, Winnebago.

Lixus tricristatus Chittenden. Proc. U. S. N. M., 77, Art. 18, 1930, p. 12.

County records: Dickinson, Johnson, Montgomery, Story.
The type resembles *scrobicollis*.

Lixus nitidulus Casey. Ann. N. Y. Acad. Sci., 6, 1891, p. 210.

County records: Story.

Reported by Hendrickson ('30).

Lixus terminalis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 157.

County records: Bremer, Clayton, Des Moines, Fayette, Fremont, Henry, Jefferson, Keokuk, Mills, Page, Story, Van Buren, Washington.

Lixus planicollis Chittenden. Proc. U. S. N. M., 77, Art. 18, 1930, p. 13.

County records: Johnson.

Lixus flexipennis Chittenden. Proc. U. S. N. M., 77, Art. 18, 1930, p. 15.

County records: Dickinson.

Lixus buchanani Chittenden. Proc. U. S. N. M., 77, Art. 18, 1930, p. 16.

County records: Dickinson.

Type locality—Lake Okoboji, Iowa.

Sternechus paludatus Casey. Ann. N. Y. Acad. Sci., 8, 1895, p. 830.

County records: Story.

This specimen agrees with Casey's type. A new state record.

Baris striata (Say). Curc., 1831, p. 17.

County records: Audubon, Buena Vista, Des Moines, Dickinson, Guthrie, Hamilton, Johnson, Linn, Osceola, Palo Alto, Plymouth, Polk, Story, Winnebago, Winneshiek.

Very common on giant ragweed.

Baris umbilicata (Leconte). Proc. Acad. N. S. Phila., 1868, p. 363.

County records: Boone, Dickinson, Dubuque, Johnson, Lee, Plymouth, Story, Winneshiek.

Baris transversa (Say). Curc., 1831, p. 18.

County records: Boone, Cherokee, Henry, Iowa, Johnson, Story, Webster, Winneshiek.

The Webster county specimen is identical with Casey's *B. gravida*.

Baris callida Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 481.

County records: Greene, Henry, Story, Winneshiek.

Baris subovalis (Leconte). Proc. Ac. Nat. Sci., Phila., 1868, p. 363.

County records: Story.

Baris dolosa Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 490.

County records: Bremer, Buena Vista, Cherokee, Clayton, Des Moines, Dickinson, Henry, Jackson, Jefferson, Johnson, Lee, Linn, Louisa, Lucas, Plymouth, Story, Wapello, Winneshiek.

Casey's type male specimen is from Keokuk, Iowa (Casey '92).

Baris xanthii Pierce. Ent. News, 18, 1907, p. 379.

County records: Clayton, Floyd, Henry, Jefferson, Scott, Union.

Baris tumescens (Leconte). Proc. Acad. N. S. Phila., 1868, p. 362.

County records: Story, Delaware.

Baris interstitialis (Say). Jour. Ac. N. S. Phila., 3, 1824, p. 314.

County records: Cherokee, Dickinson, Lee, Plymouth, Sioux, Story, Winneshiek.

Baris deformis Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 496.

County records: Boone, Clinton, Des Moines, Dickinson, Emmet, Henry, Jefferson, Johnson, Story.

Baris confinis (Leconte). Proc. Acad. N. S. Phila., 1868, p. 362.

County records: Buena Vista, Chariton, Cherokee, Clayton, Delaware, Dickinson, Henry, Jefferson, Lee (?), Linn, Lucas, Plymouth, Story.

Baris subsimilis Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 499.

County records: Lucas, Story.

Baris socialis Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 499.

County records: Bremer, Clayton, Lee, Plymouth, Story, Washington.

Reported by Blatchley ('16).

Baris aperta Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 500.

County records: Dallas, Dickinson, Henry, Jackson, Lee, Linn, Louisa, Palo Alto, Page, Plymouth, Story, Van Buren, Webster, Winneshiek.

Baris aerea (Boheman). Schoenh. Curc., 8, (1), 1844, p. 141.

County records: Appanoose, Buena Vista, Clayton, Dubuque, Henry, Story.

Baris novella Casey. Mem., 9, 1920, p. 337.

County records: Henry, Story.

Cosmobaris scolopacea (Germar). Ins. sp. nov., 1824, p. 202.

County records: Butler, Clayton, Louisa, Woodbury.

These records include the species described by Hayes as *C. squamigera* and *C. sionilli*, which are to be treated as synonyms.

Stictobaris cribrata (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 296.

County records: Plymouth.

A new state record.

Glyptobaris rugicollis (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 297.

County records: Henry.

- Onychobaris millepora** Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 526.
County records: Plymouth, Story, Winneshiek.
- Onychobaris subtonsa** Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 295.
County records: Bremer, Clayton, Henry, Plymouth, Story, Winneshiek.
Collected in Plymouth county in association with *O. millepora*.
- Onychobaris pectorosa** Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 295.
County records: Johnson, Plymouth, Story.
Reported from Johnson county by Wickham ('11).
- Madarellus undulatus** (Say). Jour. Ac. N. S. Phila., 3, 1824, p. 315.
County records: Henry, Johnson, Monroe, Story.
- Madarellus inconstans** Casey. Mem., 9, 1920, p. 354.
County records: Lee (?).
Described by Casey from Iowa. It is perhaps only a variety.
- Aulobaris nasuta** (Leconte). Proc. Ac. N. S. Phila., 1868, p. 295.
County records: Davis, Lee (?), Muscatine, Washington.
- Aulobaris pusilla** (Leconte). Proc. Ac. N. S. Phila., 1868, p. 363.
County records: Ringgold, Washington.
- Aulobaris scolopax** (Say). Curc., 1831, p. 26.
County records: Van Buren.
This single specimen is in the U. S. N. M. collection.
- Aulobaris ibis** (Leconte). Proc. Ac. N. S. Phila., 1868, p. 365.
County records: Johnson, Story.
- Aulobaris dux** Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 546.
County records: Wapello.
- Pseudobaris farcta** (Leconte). Proc. Ac. N. S. Phila., 1868, p. 362.
County records: Johnson.
- Pseudobaris sobrina** Blatchley. Rhyn. N. E. U. S., 1916, p. 373.
County records: Henry.
- Pseudobaris angusta** (Leconte). Proc. Ac. N. S. Phila., 1868, p. 363.
County records: Clayton, Decatur, Dickinson, Henry, Jefferson, Linn, Story.
- Pseudobaris nigrina** (Say). Curc., 1831, p. 26.
County records: Clayton, Des Moines, Dickinson, Henry, Linn, Story, Washington, Winnebago.

Trichobaris trinotata (Say). Curc., 1831, p. 17.

County records: Fayette, Henry, Jasper, Jefferson, Johnson, Muscatine, Story.

This is the "potato stalk borer."

Pachygeraeus laevirostris (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 309.

County records: Buchanan, Des Moines.

Centrinaspis picumnus (Herbst). Nat. Ins., 7, 1797, p. 30.

County records: Davis, Des Moines, Henry, Johnson, Muscatine, Story, Van Buren, Washington.

Centrinaspis perscillus (Gyllenhal). Schoenh. Curc., 3, (2), 1836, p. 762.

County records: Buena Vista, Dickinson, Henry, Pocahontas, Polk, Poweshiek, Story, Warren.

Centrinaspis perscitus (Herbst). Natursyst., 7, 1797, p. 28.

County records: Henry, Scott.

Centrinaspis penicellus (Herbst). Natursyst., 7, 1797, p. 29.

County records: Cherokee, Crawford, Dickinson, Fremont, Linn, Palo Alto, Plymouth, Pottawattamie, Sioux, Story.

Centrinaspis falsus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 315.

Reported by Wickham ('11).

Odontocorynus scutellum-album (Say). Curc., 1831, p. 21.

County records: Bremer, Dickinson, Iowa, Johnson, Louisa, Story, Winneshiek.

Odontocorynus iowensis Csy. Mem., 9, 1920, p. 428.

County records: Dickinson, Johnson, Winneshiek.

The type specimen is from Johnson County.

Odontocorynus denticornis Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 597.

County records: Clayton, Dickinson, Henry, Jasper, Kossuth, Plymouth, Page, Pocahontas, Story, Winnebago, Woodbury.

Odontocorynus rufobrunneus Casey. Mem., 9, 1920, p. 413.

County records: Bremer.

Compared with U. S. N. M. material and Casey's type.

Odontocorynus salebrosus Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 598.

County records: Dickinson, Hancock, Henry, Humboldt, Iowa, Sioux, Sioux, Story, Warren, Winnebago.

Centrinopus alternatus Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 602.

County records: Cherokee, Dallas, Decatur, Henry, Keokuk, Story, Winnebago, Winneshiek, Woodbury.

A new state record.

- Nicentrus lineicollis** (Boheman). Schoenh. Curc., 8, (1), 1844, p. 221.
County records: Boone, Linn.
- Nicentrus ingenuus** Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 610.
County records: Jefferson, Story.
- Nicentrus simulans** Casey. Mem., 9, 1920, p. 449.
County records: Fremont, Linn.
Described from Iowa by Casey ('20).
- Nicentrus vacunalis** Casey. Mem., 9, 1920, p. 450.
County records: Fremont.
The type is from Riverton, Iowa.
- Centrinites strigicollis** Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 616.
County records: Jefferson, Johnson, Story.
- Anacentrus (Oligolochus) bracata** (Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 627.
County records: Bremer, Henry, Johnson, Linn, Story.
- Anacentrinus deplanata** (Casey). Ann. N. Y. Acad. Sci., 6, 1892, p. 630.
County records: Bremer, Dallas, Hancock, Lee, Plymouth, Story.
Found in the axils of mullein leaves where they overwinter in the adult form.
- Anacentrinus oblitus** (Casey). Ann. N. Y. Acad. Sci., 6, 1892, p. 634.
County records: Cherokee, Dickinson.
- Sibariops confusa** (Boheman). Schoenh. Curc., 3, (2), 1836, p. 740.
County records: Des Moines, Johnson, Jones, Lucas, Story, Washington.
A new state record.
- Sibariops confinis** (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 317.
County records: Henry, Johnson, Lee (?), Story, Washington.
- Sibariops civica** Casey. Mem., 9, 1920, p. 491.
County records: Lee (?).
The type is described from Iowa.
- Cylindrida prolixa** (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 317.
County records: Dickinson, Johnson, Pocahontas, Pottawattamie, Story, Washington.
- Dirabius rectirostris** (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 315.
County records: Buchanan, Buena Vista, Dickinson, Hancock, Johnson, Jones, Palo Alto, Story, Winnebago.
- Idiostethus tubulatus** (Say). Curc., 1831, p. 20.
County records: Bremer, Delaware, Dickinson, Emmet, Jefferson, Jones, Kossuth, Linn, Muscatine, Story, Washington.

Idiostethus puncticollis Casey. Mem., 9, 1920, p. 500.

County records: Johnson, Story.

Probably a synonym of *tubulatus*, of which Casey had no specimen.

Idiostethus subcalvus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 622.

County records: Lee.

One specimen in the U. S. N. M. collection determined by Buchanan.

Haplostethops ellipsoideus (Casey). Ann. N. Y. Acad. Sci., 6, 1892, p. 652.

County records: Delaware, Henry, Lee (?), Story.

Haplostethops caviventrus Blatchley. Jour. N. Y. Ent. Soc., 30, 1922, p. 119.

County records: Calhoun, Dickinson, Johnson.

Determined as such in the U. S. N. M. collection.

Idiostethus dispersus Casey. Ann. N. Y. Acad. Sci., 6, 1892, p. 652.

County records: Henry, Story.

Stethobaris ovata (Lec). Proc. Ac. N. S., 1858, p. 363.

County records: Story.

Zaglyptus striatus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 237.

County records: Story.

A new state record.

Oomorphidius erasus (Leconte). Trans. Am. Ent. Soc., 7, 1860, p. 217.

County records: Fremont.

Catapastus conspersus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 318.

County records: Clayton, Johnson, Lee, Lucas, Plymouth, Story.

Barinus cribricollis (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 422.

County records: Story.

Barinus squamolineatus Casey. Calif. Acad. Sci., 1886, p. 256.

County records: Allamakee, Buchanan, Des Moines, Johnson, Lee, Palo Alto, Story.

Barinus linearis (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 422.

County records: Johnson.

Barilepton filiforme Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 319.

County records: Dickinson.

Eunyssobia echidna (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 320.

Reported by Wickham ('11) without locality.

Gelus oculatus (Say). Jour. Acad. Nat. Sci., Phila., 3, 1824, p. 308.

County records: Clayton, Henry, Johnson, Lee, Louisa, Story, Van Buren, Washington.

Cylindrocopturus binotatus (Lec.). Proc. Am. Phil. Soc., 15, 1876, p. 263.

County records: Cherokee, Dickinson, Plymouth, Story, Winneshiek.

Cylindrocopturus adpersus (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 262.

County records: Winneshiek.

Cylindrocopturus operculatus (Say). Jour. Ac. N. S. Phila., 3, 1824, p. 308.

County records: Cherokee, Decatur, Dickinson, Fremont, Keokuk, Plymouth, Story.

Cylindrocopturus sparsus (Casey). Ann. N. Y. Acad. Sci., 9, 1897, p. 673.

County records: Dickinson, Iowa.

Cylindrocopturus nanulus (Lec.). Proc. Am. Phil. Soc., 15, 1876, p. 261.

County records: Lee (?).

Reported from Iowa by Casey ('97).

Cylindrocopturus quercus (Say). Curc., 1831, p. 20.

County records: Blackhawk, Linn, Johnson, Washington.

Acoptus suturalis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 264.

County records: Howard.

Psomus politus Casey. Col. Not. 4, 1892, p. 459.

County records: Dickinson.

A new state record.

Mononychus vulpeculus (Fabricius). Syst. El., 2, 1801, p. 450.

County records: Dickinson, Johnson.

Craponius inequalis (Say). Curc., 1831, p. 20.

County records: Story.

A new record.

Acanthoscelis curtus (Say). Curc., 1831, p. 29.

County records: Story.

A new state record.

Acanthoscelis acephalus (Say). Jour. Ac. N. S. Phila., 3, 1824, p. 309.

County records: Clayton, Monroe, Story, Union.

Auleutes asper (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 270.

County records: Clarke.

Auleutes nebulosus (Lec.). Proc. Am. Phil. Soc., 15, 1876, p. 271.

County records: Winnebago.

Hypocoeliodes wickhami Dietz. Trans. Am. Ent. Soc., 23, 1896, p. 416.

County records: Calhoun.

Acollodes saltoides Dietz. Trans. Am. Ent. Soc., 23, 1896, p. 416.

County records: Bremer, Dickinson, Story.

Coeliodes flavicaudis Boheman. Schoenh. Curc., 8, (1), 1844, p. 397.

County records: Boone, Butler, Cherokee, Clay, Clayton, Dickinson, Jefferson, Linn, Plymouth, Scott, Story, Van Buren, Winnebago.

Ceutorhynchus rapae Gyll. Schoenh. Curc., 1837, p. 547.

County records: Boone, Des Moines, Dickinson, Harrison, Henry, Howard, Johnson, Lee, Palo Alto, Plymouth, Story, Winneshiek, Woodbury.

Known as the "cabbage curculio."

Ceutorhynchus sericans Lec. Proc. Am. Phil. Soc., 15, 1876, p. 275.

Reported from Iowa without record by Wickham ('11).

Ceutorhynchus sulcipennis Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 274.

County records: Benton, Blackhawk, Boone, Cedar, Deleware, Fremont, Hancock, Hardin, Iowa, Johnson, Kossuth, Linn, Louisa, Lucas, Mills, Palo Alto, Story, Webster, Winnebago.

Ceutorhynchus cyanipennis Germar. Ins. Spec. Nov., 1, 1824, p. 235.

County records: Des Moines, Dickinson, Emmet, Humboldt, Johnson, Osceola, Story.

Ceutorhynchus neglectus Blatch. Rhyn. N. E. U. S., 1916, p. 447.

County records: Boone, Cass, Cherokee, Dickinson, Johnson, Plymouth, Story, Winneshiek.

Ceutorhynchus pauxillus Dtz. Trans. Am. Ent. Soc., 23, 1896, p. 442.

County records: Des Moines, Plymouth.

A new state record.

Ceutorhynchus squamatus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 277.

County records: Cedar, Cherokee, Des Moines, Dickinson.

Ceutorhynchus septentrionalis Gyllenhal. Schoenh. Curc., 4, (1), 1837, p. 492.

County records: Calhoun, Henry, Johnson, Lee, Mahaska, Story.

Ceutorhynchus puberulus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 279.

County records: Buchanan, Dickinson, Johnson.

Ceutorhynchus zimmermanni Gyllenhal. Schoenh. Curc., 1837, p. 492.

County records: Johnson.

Reported by Wickham ('11).

Perigaster lituratus (Dietz). Trans. Am. Ent. Soc., 23, 1896, p. 457.

An Iowa paratype of Buchanan's *longirostris*, a synonym of this

species, is in the U. S. N. M. collection, Cat. No. 43532. It was collected by Shimek.

Perigaster cretura (Herbst). *Natursyst.*, 7, 1797, p. 70.

County records: Johnson, Story.

Phytobius sulcicollis Fahreus. *Schoenh. Curc.*, 7, (2), 1843, p. 346.

County records: Blackhawk, Bremer, Clayton, Dickinson, Henry, Johnson, Linn, Louisa.

Phytobius squamosus (Leconte). *Proc. Am. Phil. Soc.*, 15, 1876, p. 281.

County records: Johnson.

Mecopeltus fuliginosus Dietz. *Trans. Am. Ent. Soc.*, 23, 1896, p. 497.

County records: Linn, Story.

Rhinoncus pericarpus (Linn.). *Syst. Nat.*, 1758, p. 380 (nec Fab. 01-451, Leng).

County records: Clay, Dickinson, Fremont, Hancock, Harrison, Iowa, Jefferson, Kossuth, Linn, Mills, Palo Alto, Pottawattamie, Winnebago.

Recorded by Wickham under the name of *R. occidentalis* Dtz., a synonym.

Rhinoncus pyrrhopus Boheman. *Schoenh. Curc.*, 8, (2), 1845, p. 172.

County records: Adair, Adams, Benton, Cerro Gordo, Floyd, Henry, Ida, Jackson, Jefferson, Johnson, Jones, Linn, Polk, Pottawattamie, Plymouth, Sac, Story, Winneshiek.

Rhinoncus longulus Leconte. *Proc. Am. Phil. Soc.*, 15, 1876, p. 284.

County records: Boone, Cedar, Clinton, Louisa, Mahaska, Story.
Not reported by Wickham.

Amalus haemorrhous (Herbst). *Natursyst.*, 6, 1795, p. 399.

County records: Cherokee, Hancock, Humboldt, Plymouth, Winneshiek.

First records from Iowa by Hendrickson ('30).

Litodactylus griseomicans (Schwarz). *Proc. Ent. Soc. Wash.*, 2, 1892, p. 165.

County records: Dickinson, Hamilton.

Eubrychiopsis lecontei (Dietz). *Trans. Am. Ent. Soc.*, 23, 1896, p. 475.

County records: Dickinson.

Conotrachelus juglandis Leconte. *Proc. Am. Phil. Soc.*, 15, 1876, p. 226.

County records: Clayton, Story.

Conotrachelus albicinctus Leconte. *Proc. Am. Phil. Soc.*, 15, 1876, p. 226.

County records: Dickinson, Story.

Conotrachelus seniculus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 227.

County records: Appanoose, Clayton, Emmet, Henry, Howard, Jefferson, Johnson, Keokuk, Louisa, Madison, Page, Story, Union, Wapello.

Conotrachelus elegans (Say). Curc., 1831, p. 18.

County records: Bremer, Story.

Conotrachelus nivosus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 229.

County records: Cherokee, Plymouth, Story.

Conotrachelus crataegi Walsh. Proc. Bost. Soc. Nat. Hist., 9, 1864, p. 311.

County records: Fayette, Henry, Story.

Known as the "quince curculio."

Conotrachelus naso Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 231.

County records: Buchanan, Clayton, Henry, Johnson, Story.

The Johnson county specimen was labelled *affinis* by Wickham.

Conotrachelus posticatus Boh. Schoenh. Curc., 4, (2), 1837, p. 406.

County records: Dickinson, Johnson, Story.

Conotrachelus recessus (Casey). Can. Ent., 42, 1910, p. 130.

County records: Story.

Described by Casey as *Loceptes recessus* and discovered by Buchanan to be a *Conotrachelus* species. It is extremely small.

Conotrachelus geminatus Dejean. Cat. Col. Dej., 3, 1837, p. 322.

County records: Des Moines, Iowa, Johnson, Muscatine, Story.

Dr. Harris found them burrowing in stems of *Polygonum nitella*.

Conotrachelus cribricollis (Say). Curc., 1831, p. 28.

County records: Dallas, Muscatine, Story.

Conotrachelus anaglypticus (Say). Curc., 1831, p. 18.

County records: Bremer, Clay, Dickinson, Henry, Johnson, Kosuth, Story, Winneshiek.

Conotrachelus leucophaeatus Fahreus. Schoenh. Curc., 4, (1), 1837, p. 417.

County records: Cherokee, Story, Woodbury.

Ryssematus lineaticollis (Say). Jour. Ac. Nat. Sci., Phil., 3, 1824, p. 313.

County records: Appanoose, Des Moines, Dickinson, Guthrie, Hamilton, Howard, Humboldt, Lee, Linn, Muscatine, Plymouth, Story, Winneshiek.

Casey's *R. grandicollis* records are included under this species, his type being considered as only a large specimen of *lineaticollis*.

- Ryssematus aequalis** Horn. Proc. Am. Phil. Soc. 13, 1873, p. 464.
County records: Cherokee, Dickinson, Henry, Jefferson, Johnson, Plymouth, Wapello, Winneshiek.
- Ryssematus palmacollis** (Say). Curc., 1831, p. 16.
County records: Plymouth.
A tentative determination.
- Chalcodermus aeneus** Boheman. Schoenh. Curc., 4, (1), 1837, p. 388.
County records: Boone.
- Chalcodermus collaris** Horn. Proc. Am. Phil. Soc., 13, 1873, p. 467.
County records: Story.
- Microhyus setiger** Lec. Proc. Am. Phil. Soc., 15, 1876, p. 238.
County records: Story.
- Tyloderma foveolata** (Say). Desc. Curc., 1831, p. 19.
County records: Clay, Clayton, Henry, Ida, Johnson, Jones, Osceola, Page, Scott, Warren, Winneshiek.
- Tyloderma fragariae** (Riley). 3rd Ann. Rept. Ins. Mo., 1871, p. 42.
County records: Blackhawk, Johnson, Lee, Story, Wapello.
This is the "strawberry crown-borer."
- Tyloderma variegata** (Horn). Proc. Am. Phil. Soc., 13, 1873, p. 468.
County records: Palo Alto.
- Tyloderma aerea** (Say). Curc., 1831, p. 29.
County records: Henry, Howard, Story.
- Tyloderma aerea** var. **nigra** Casey. Bull. Brook. Ent. Soc., 7, 1884, p. 56.
County records: Story.
- Eurhoptus pyriformis** Lec. Proc. Am. Phil. Soc., 15, 1876, p. 245.
County records: Boone, Johnson.
- Acalles pectoralis** Lec. Proc. Am. Phil. Soc., 15, 1876, p. 245.
County records: Story.
- Cryptorhynchus parochus** (Herbst). Jablonsky, Nat. Ins., 1797, p. 55.
County records: Henry, Story.
- Cryptorhynchus bisignatus** Say. Curc., 1831, p. 19.
County records: Johnson, Story.
- Cryptorhynchus pumilus** Boheman. Schoenh. Curc., 4, (1), 1837, p. 122.
An Iowa specimen from the Wickham collection in the U. S. National Museum collection.

Cryptorhynchus fuscatus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 251.

County records: Boone, Johnson.

Cryptorhynchus obliquus Say. Curc., 1831, p. 28.

County records: Johnson.

Cryptorhynchus obtentus (Hbst.). Jablonsky, Nat. Ins., 7, 1797, p. 38.

County records: Story.

Cryptorhynchus tristis Lec. Proc. Am. Phil. Soc., 15, 1876, p. 255.

County records: Johnson, Woodbury.

Thecesternus humeralis (Say). Jour. Acad. Nat. Hist., Phila., 5, 1826, p. 254.

County records: Common throughout the state.

Acamptus echinus Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 445 (nec 95-837, Leng).

County records: Mahaska.

Dryophthorus americanus Bedel. Rhynchophora, 1885, p. 192.

County records: Henry, Johnson, Jones.

This is the *D. corticalis* of Say, which name was preoccupied in the genus.

Cossonus platalea Say. Curc., 1831, p. 24.

County records: Henry, Johnson, Story.

Cossonus subareatus Boheman. Schoenh. Curc., 8, (2), 1845, p. 266.

County records: Wapello.

Cossonus impressifrons Boheman. Schoenh. Curc., 4, (2), 1838, p. 1001.

County records: Henry, Jones, Lee, Monroe.

Cossonus corticola Say. Curc., 1831, p. 24.

County records: Henry.

Hexarthrum ulkei Horn. Proc. Am. Phil. Soc., 18, 1873, p. 446.

County records: Des Moines.

Rhyncolus carinatus Blatchley. Rhynch. N. E. U. S., 1916, p. 545.

County records: Story.

A new record for the state.

Stenoscelis brevis (Boheman). Schoenh. Curc., 8, (2), 1845, p. 282.

A Wickham specimen with the antennal funicle 6-jointed instead of 7-jointed is in the U. S. N. M. collection.

Rhodobaenus 13-punctata (Illiger). N. Mag. Lieb. Ent., 5, 1794, p. 613.

Common on ragweed and cocklebur throughout the state.

Rhodobaenus 13-punctata var. **pulchellus** (Gyllenhal). Schoenh. Curc., 4, (2), 1838, p. 941.

Also common throughout the state.

Calendra aequalis (Gyllenhal). Schoenh. Curc., 4, (2), 1838, p. 941.

County records: Boone, Clayton, Decatur, Dickinson, Harrison, Henry, Kossuth, Lyons, Muscatine, Pottawattamie.

A common species and the largest *Calendra* in Iowa.

Calendra ochreus (Leconte). Proc. Acad. Nat. Sci., Phila., 1858, p. 80.

County records: Johnson, Pottawattamie, Story.

This is in part a synonym of *aequalis*, according to Blatchley ('16), and perhaps the above records are such.

Calendra striatipennis (Chittenden). Jour. N. Y. Ent. Soc., 14, 1906, p. 180.

County records: Dickinson, Story, Winnebago.

Calendra pertinax (Olivier). Ent., 5, 1807, p. 90.

County records: Dickinson, Harrison, Howard, Johnson, Story, Washington.

Calendra setiger (Chittenden). Proc. Ent. Soc., Wash., 7, 1906, p. 55.

County records: Decatur, Story.

Calendra robusta (Horn). Proc. Am. Phil. Soc., 13, 1873, p. 419.

County records: Dickinson.

C. multilineatus Satterthwait is a synonym of this species. A paratype from the Shimek collection is an Iowa specimen.

Calendra costipennis (Horn). Proc. Am. Phil. Soc., 13, 1873, p. 420.

County records: Clayton, Dickinson, Pottawattamie.

Calendra soltaui (Chittenden). Proc. Ent. Soc. Wash., 7, 1906, p. 178.

County records: Johnson, Story (?).

Calendra parvula (Gyllenhal). Schoenh. Curc., 4, (2), 1838, p. 961.

County records: Cass, Henry, Pocahontas, Story.

Very common throughout the state.

Calendra minima (Hart). 16th Rept. Ill. St. Ent., 1890, p. 65.

County records: Henry.

Calendra retusa (Gyllenhal). Schoenh. Curc., 1838, 4, (2), p. 949.

County records: Story.

Calendra destructor (Chittenden). Proc. Ent. Soc., Wash., 7, 1906, p. 174.

County records: Henry, Monroe.

Calendra zaeae (Walsh). Pract. Ent., 2, 1867, p. 117.

County records: Des Moines, Henry, Johnson, Louisa, Monroe, Story, Van Buren.

Calendra scoparia (Horn). Proc. Am. Phil. Soc., 13, 1873, p. 424.

County records: Humboldt, Story.

Calendra callosa (Olivier). Ent., 5, 1807, p. 92, pl. 28, fig. 416.

County records: Clayton, Monroe, Sioux, Story.

Calendra melanocephala (Fabricius). Syst. Eleuth., 2, 1801, p. 435.

County records: Boone, Des Moines, Henry, Humboldt, Jasper, Johnson, Jones, Scott, Story, Washington.

Calendra sayi (Gyllenhal). Schoenh. Curc., 4, (2), 1838, p. 943.

County records: Dickinson, Story.

Calendra venatus (Say). Curc., 1831, p. 22.

County records: Clayton, Henry, Johnson, Keokuk, Plymouth, Story.

Calendra vestitus (Chittenden). Proc. Ent. Soc. Wash., 6, 1904, p. 134.

County records: Henry.

Calendra incongrua (Chittenden). Proc. Ent. Soc. Wash., 7, 1905, p. 61.

Satterthwait ('31) reports it from Iowa.

Calendra robustior (Chittenden). Proc. Ent. Soc. Wash., 7, 1905, p. 62.

County records: Humboldt.

Calendra robustior var. **costifer** Chitt. Proc. Ent. Soc. Wash., 31, 1924, p. 152.

County records: Dickinson.

Paratype No. 26895 U. S. N. M. collection.

Calendra jugosa Chittenden. Proc. Ent. Soc. Wash., 31, 1924, p. 151.

Type No. 26892 U. S. N. M. collection. It was described without locality. It is an Iowa specimen from the Wickham collection.

Sitophilus granaria (L.). Syst. Nat., (10th ed.), 1758, p. 378.

This is the "granary weevil" and is generally distributed throughout the state.

Sitophilus oryzae (L.). Amoen. Acad., 6, 1763, p. 395.

The "rice weevil" has been reported from various parts of the state.

SCOLYTOIDEA

Family Scolytidae

Scolytus quadrispinosus Say. Proc. Ac. Nat. Sci., Phila., 3, 1824, p. 323.

County records: Johnson, Story (?).

Scolytus muticus Say. Proc. Ac. Nat. Sci., Phila., 3, 1824, p. 323.

County records, Story.

Scolytus rugulosus Ratz. Die Forstinsekten, etc., 1837, p. 187.

County records: Wapello.

Chramesus hicoriae Leconte. Proc. Ac. Nat. Sci., Phila., 1868, p. 168.

County records: Johnson, Story.

Phthorophloeus limnaris (Harr.). Rept. Inj. Ins., ed. 2, 1852, p. 78.

County records: Johnson.

Phthorophloeus frontalis (Olivier). Ent. 4, 1795, p. 13.

County records: Des Moines.

Leperisinus aculeatus (Say). Proc. Ac. N. S., Phila., 1824, p. 322.

County records: Des Moines, Johnson, Story.

Hylurgopinus rufipes (Eich.). Desc. of Scolytidae, 1868, p. 147.

County records: Johnson.

Pterocyclon fasciatum Say. Proc. Ac. N. S., Phila., 5, 1825, p. 255.

County records: Story.

Monarthrum mali (Fitch). 3rd Ann. Rept. N. Y. St. Agr. Soc., 1856, p. 326.

County records: Johnson.

Xyloterinus politus (Say). Proc. Acad. Nat. Sci., Phila., 1826, p. 256.

County records: Story.

Trypodendron retusum (Leconte). Proc. Acad. Nat. Sci., Phila., 1868, p. 158.

County records: Johnson.

Pseudopityophthorus minutissimus (Zimmerman). Syn. Scholytidae, 1868, p. 143.

County records: Johnson.

Pityophthorus rhois Swaine. Can. Bark. Beetles, 1, 1917, p. 26.

County records: Story.

Pityophthorus puberulus (Leconte). Proc. Acad. Nat. Sci., Phila., 1868, p. 157.

Pityogenes carinulatus (Leconte). Trans. Am. Ent. Soc., 5, 1874, p. 70.

County records: Story.

Pityogenes knechteli Swaine. Can. Bark. Beetles, 2, 1918, p. 106.

County records: Plymouth.

Ips calligraphus (Germar). Ins. sp. nov., 1, 1824, p. 461.

County records: Story.

Ips pini (Say). Jour. Acad. Nat. Sci., Philaa., 5, 1826, p. 257.

County records: Johnson (?).

Ips. n. sp.

A Scolytid taken at the lights by the author was unfortunately in such condition that Mr. Blackman could be certain only of the genus. Of the species, he says "Unknown to me and I do not believe it has been described from North America."

Orthotomicus caelatus (Eich). Desc. Scolytidae, 1867, p. 402.

County records: Story.

Xyleborus pubescens Zimmerman. Trans. Am. Ent. Soc., 1, 1868, p. 145.

County records: Johnson.

Xyleborus celsus Eich. Desc. Scolytidae, 1867, p. 400.

County records: Johnson.

Lymanator decipiens Leconte. Proc. Am. Phil. Soc., 17, 1878, p. 624

County records: Story.

Pseudothysanoes drakei Blackm. Miss. Agri. Exp. Sta. Tech. Bull., 9, 1920. p. 48.

County records: Story.

SPECIES OF PROBABLE OCCURRENCE IN IOWA

Apion obsoletum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 44.

This species is reported from Michigan, Missouri and Dakota. Blatchley ('16) adds Nebraska.

Apion extensum Smith. Trans. Am. Ent. Soc., 11, 1874, p. 47.

Originally reported from Dakota. Blatchley reports it from Indiana.

Trachyphloeus asperatus Boheman. Schoenh. Curc., 7, (1), p. 116 (nec 1844, Leng).

Blatchley gives it doubtful occurrence throughout the middle states.

Lepyrus palustris (Scopoli). Ent. Carn., 1763, p. 33.

Blatchley gives it as occurring in Indiana, New York, to Wisconsin, Colorado and Manitoba, south to Louisiana.

Hyperodes rotundicollis (Dietz). Trans. Am. Ent. Soc., 16, 1889, p. 44.

Blatchley states that it ranges from N. Y. to Nebraska and Colorado, south to Texas.

Pseudanthonomus seriesetosus Dietz. Trans. Am. Ent. Soc., 18, 1891, p. 251.

Blatchley gives D. C., Mich, and Nebr. as its range.

Ampelogypter longipennis Casey. Ann. N. Y. Ac. Sci., 6, 1892, p. 549.

It ranges from Penn. and Md. to Nebr., according to Blatchley.

Barilepton quadricolle Lec. Proc. Am. Phil. Soc., 15, 1876, p. 423.

Blatchley records it from Mich., s. Ill., and Nebr.

Cylindrocopturus longulus (Lec). Proc. Am. Phil. Soc., 15, 1876, p. 263.

Reported from New England to Canada, Utah and California.

Auleutes tenuipes (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 270.

Blatchley gives its range from New England and Canada to Mo., Ga., and Texas.

Auleutes cruralis (Leconte). Proc. Am. Phil. Soc., 15, 1876, p. 270.

Blatchley records it from Newfoundland and L. Superior to Utah, south to Pa. and Texas.

Ceutorhynchus decipens Leconte. Proc. Am. Phil. Soc., 15, p. 275.

Canada and New England to Mich., south to N. C. and west to Kansas, Utah, Colo., and Calif., is the range given by Blatchley.

Ceutorhynchus ovalis Dietz. Trans. Am. Ent. Soc., 23, 1896, p. 438.

Its type localities are Illinois and Minnesota.

Calendra maidis (Chittenden). Proc. Ent. Soc. Wash., 7, 1905, p. 59.

Reported from Mich., Kansas, Texas and Alabama.

Calendra inaequalis (Say). Curc., 1831, p. 23.

Satterthwait ('31) reports in in the larval form from Ala., Fla., Minn., Md., N. Y., Pa., and S. C.

Calendra cariosa (Olivier). Ent. Carn., 1807, p. 27.

Larvae were reported from Ill., Mo., Nebr., and Kansas.

Calendra oblita (Lec). Proc. Am. Phil. Soc., 15, 1876, p. 425.

Larvae were reported from Mich., Wisc., Kans., Md., Tex., and Ariz.

DOUBTFUL IOWA RHYNCHOPHORA RECORDS

Apion floridanum Smith. Trans. Am. Ent. Soc., 11, 1884, p. 49.

Recorded by Wickham ('11) from Iowa. Undoubtedly an incorrect determination by Smith.

Listronotus callosus Lec. Proc. Am. Phil. Soc., 15, 1876, p. 130.

Reported from Ames and Eldora by Wickham ('11). Its range is much more southern.

Anthonomus nubilus Leconte. Proc. Am. Phil. Soc., 15, 1876, p. 205.

Reported from Iowa City by Wickham ('11). A specimen in his collection is really Fall's *nubiloides*.

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